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THE EARLY DEVELOPMENT OF THE PIGEON'S EGG, WITH ESPECIAL REFERENCE TO POLYSPERMY AND THE ORIGIN OF THE PERIBLAST NUCLEI.[1]

BY

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With 54 Figures.

CONTENTS.

[1]A dissertation submitted to the faculty of the Ogden Graduate School of Science, University of Chicago, in candidacy for the degree of Doctor of Philosophy.

Introduction.

The history of the early development of the bird's egg has been obscure because of the difficulty of obtaining abundant material in a close series of consecutive stages. On account of the regular egg laying habits of pigeons and because they breed readily in confinement, they offer invaluable material to the student of bird embryology.

The pigeon lays two eggs. The first is laid about 4.30 or 5.00 o'clock in the afternoon. About eight o'clock in the evening of the same day the second egg leaves the ovary and is fertilized at that time. It is laid about 1.00 or 1.30 p. m. of the second day following the time of laying of the first egg; that is, it is laid about forty-one hours after fertilization. It is evident that after the first egg is laid, the pigeon may be killed and the second egg obtained in any desired stage.

These facts were published by Dr. E. H. Harper ('04) in his thesis on "The Fertilization and Early Development of the Pigeon's Egg." In regard to ovulation, Dr. Harper says (p. 352), "In all cases observed, this has taken place between seven and nine o'clock." In this paper I shall refer to eight o'clock in the evening as the approximate time of fertilization, although the exact time for any particular egg is not known.

Some of the results of my research were published in a preliminary paper in the Biological Bulletin, October, 1907.

For this research, one hundred and forty-four eggs have been obtained, covering the period required for the egg to pass through the oviduct. Of these, there is an egg for every hour, with more abundant material at critical stages. The present thesis refers especially to the first fifteen hours after fertilization. Problems in later oviducal development are reserved for publication at some future time.

My thanks are due to Professor Whitman and other members of the Department of Zoölogy for a fellowship and an assistantship, which has made it possible for me to carry on this research. Professor F. R. Lillie, who suggested the problem, has followed the work with helpful interest, and I am particularly indebted to him. Professor W. L. Tower has given indispensable help in the technique of photography, and Professor C. M. Child has helped me with some literature. Figs. 5, 6, 9, 11 and 14 are the work of Mr. Kenji Toda.

I. Methods.

Following the methods of workers who have preceded me, the blastoderm has been killed and hardened on the yolk and the orientation marked with a bristle. Immediately after a window has been

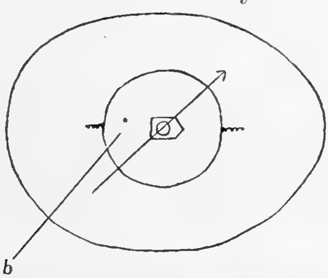


Fig. 1.—Diagram to show the method of marking the orientation. The arrow indicates the direction of the axis of the future embryo. b, bristle.

made through the shell, a bristle is inserted in the side of the yolk toward the blunt pole of the shell. Later (usually when the egg is in 70 per cent alcohol) a five-sided piece, including the blastoderm, is cut out from the yolk. One side of the five-sided area is perpendicular to the chalazal axis and is toward the large pole of the egg. Two sides are parallel to each other and to the chalazal axis, and the last two sides meet in a sharp angle pointed toward the small pole of the egg. Fig. 1 explains this orientation, the anterior side of the blastoderm being toward the point of the arrow. This five-sided block is easily seen in the paraffin cake for orientation in cutting. In some eggs there is little difference between the blunt and sharp poles of the shell, and in these the orientation is uncertain after the egg has been taken out of the oviduct. While the egg

is passing through the oviduct, the small end is directed posteriorly. I have taken the precaution to indicate by a pencil mark on the shell the orientation of the egg in the oviduct. Or, if it was obtained before the formation of the shell, the orientation was marked with a bristle inserted into the yolk before the egg was removed from the oviduct.

As for killing fluids, I have used Kleinenberg's picro-sulphuric acid (strong solution) plus ten per cent acetic acid more than any other. Flemming's fluid is good for surface views, but not for material which is to be sectioned.

Most of my material has been imbedded in paraffin according to the ordinary method, but there has been great difficulty in cutting. In the last part of my work, I have had better success with rubber paraffin—the method described by Johnston ('03). The sections have been cut usually 6 microns.

Formalin (three or five per cent) has been favorable for killing those eggs that were to be used as whole mounts. With Conklin's hæmatoxylin stain they present considerable differentiation in different regions of the blastoderm.

In photographing the eggs, an arc light has been used for illumination. Sometimes the eggs have been removed from the shell and albumen and placed in a dish of salt solution. Others have been photographed in formalin after having been removed from the egg envelopes. In a few cases the egg was left in the shell, through which a window was made in order to expose the blastoderm. Of course, when the egg is in this position, it is difficult to get sufficient light directed down into the egg and onto the blastoderm. One cannot be sure that such a photograph shows all of the accessory cleavage, and other details. The magnification was obtained by photographing through a microscope with No. 1 Leitz ocular, and No. 2 Leitz objective.

II. Distribution of Nuclei During the First Fourteen Hours After Fertilization.

In the surface view of a pigeon egg in the maturation stage, two areas are more or less distinct. They were figured by Harper ('04,

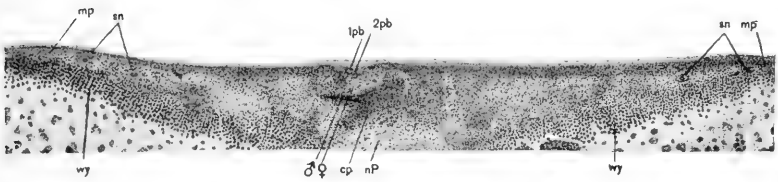


FIG. 2.—Transverse section of a pigeon's egg taken from the oviduct at 11:30 p. m., about 3½ hours after fertilization. Reference marks are the same as for Fig. 3. Leitz, 4/2. Tube length, 140 mm.

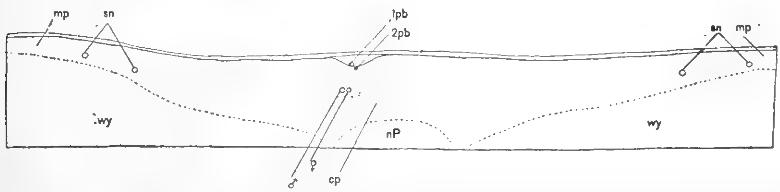


FIG. 3.—Diagram of Fig. 2. A central transverse section of pigeon's egg obtained at 11:30 p. m., 3½ hours after fertilization. The nuclei are reconstructed from seven successive sections.

1 pb, first polar body; 2 pb, second polar body; ♂, male pronucleus; ♀, female pronucleus; sn, supernumerary sperm nuclei; mp, marginal periblast; cp., central periblast; wy, white yolk; n P, nucleus of Pander.

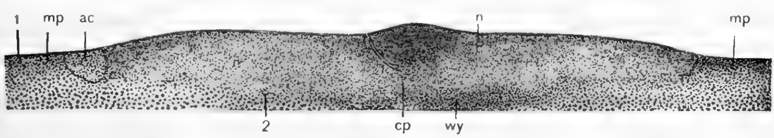


FIG. 4.—Transverse section through about the center of the blastoderm of a pigeon's egg obtained at 2:00 a. m., about six hours after fertilization. The same egg is shown in Chart II. n, nucleus of one of the blastomeres. mp, marginal periblast. cp, central periblast. wy, white yolk. ac, a cell in the accessory cleavage. 1, a sperm nucleus in the marginal periblast, and 2, a sperm nucleus in the central periblast. Leitz, 4/2. Tube length, 140 mm.

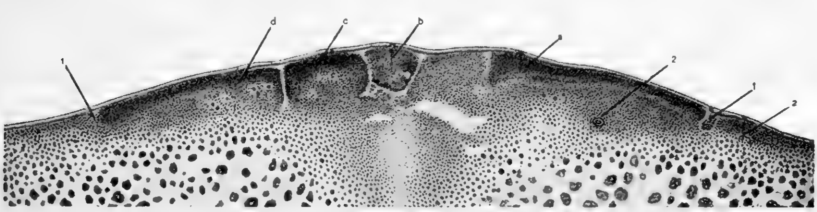


FIG. 5.—Transverse section of the pigeon egg whose surface view is shown in Fig. 37. a, b, c, d, cells of primary cleavage. 1, accessory cleavage. 2, sperm nuclei.

Fig. 6) and were described by him as follows: "The slightly oval disc has a greater diameter of 3.5 mm. It is divided into two zones quite clearly distinguished in opacity, the outer zone being due to the abrupt thinning out of the fine granular matter of the disc." These two areas are the blastodisc and the periblast. In an egg obtained at 11 p.m., about three hours after fertilization, the blastoderm was 2.96 mm. in transverse and 3.08 mm. in longitudinal diameter. These measurements were taken on the living egg. Fig. 45 shows the surface view of such an egg.

The central transverse section of another egg gives the diameter of the blastodisc about 2.5 mm. This egg was taken from the oviduct at 11.30 p.m. (three and one-half hours after fertilization). In the sections I found the male and female pronuclei and a number of supernumerary sperm nuclei. The latter had migrated peripherally from their place of entrance and occupied a circle at the inner margin of the periblast. Several nuclei were peripheral to those at the inner margin of the periblast. They are probably not nuclei of the original migration, but may be sisters to those just central to them, if we may suppose that a nuclear division has taken place since the migration into the periblast. Fig. 2 shows a central transverse section of this egg, but the nuclei were in several successive sections. See also a diagram traced from this figure, Fig. 3.

The distribution of the sperm nuclei, their disappearance, and later the distribution of the periblast nuclei are illustrated in seven charts. The figure lettered A in each chart represents the surface view of the egg. The orientation is the same for all the surface views presented in this thesis. The anterior side of the blastoderm is away from the observer, and the axis of the future embryo is in a diagonal position, as indicated in Fig. 1. These figures (A of the charts) were drawn from the living eggs in salt solution to the same scale as nearly as possible in free hand work. When a dotted circle occurs in A or B of any chart, it represents the *apparent* peripheral limit of the marginal periblast. The supernumerary sperm nuclei, and later the periblast nuclei, may migrate peripherally to this distance. How much further the cytoplasm of the periblast extends I do not know; for I cannot demonstrate the existence of

cytoplasm in the mass of yolk granules except by the presence of nuclei and except in a few places where cytoplasmic islands and strands appear. The periblastic zone, as shown by a dotted circle in the charts, appears in the surface view of the living eggs at certain

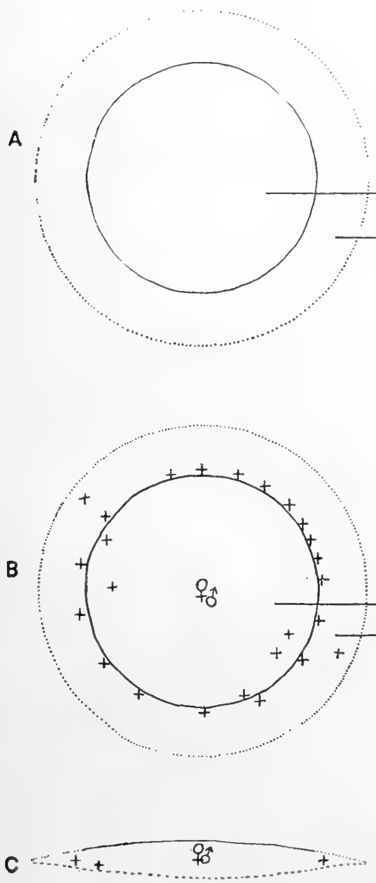


Chart I.

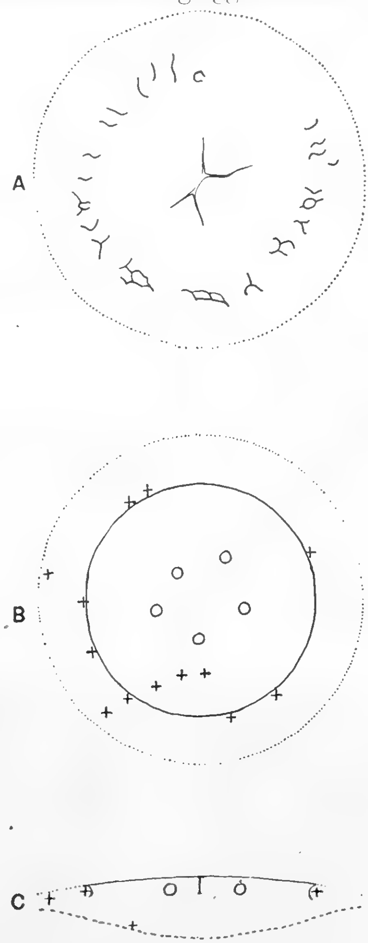


Chart II.

stages and is shown in several photographs. Fig. B is a diagram reconstructed from a study of transverse sections, and Fig. C is a diagram of a central transverse section. The sperm nuclei and the periblast nuclei represented in Fig. B of the several charts are

in superficial positions in the marginal periblast, but those within the circle bounding the blastodisc are in the region recognized as the central periblast. They are therefore deep and are not confused with the nuclei of primary cleavage. The following characters are used in the charts:

♂, male pronucleus. ♀, female pronucleus. o, primary cleavage nucleus.
θ, periblast nucleus. +, sperm nucleus. bl., blastodisc. per., periblast.

Chart I.

From a study of the sections, then, it becomes evident that before the appearance of the first cleavage plane the supernumerary sperm nuclei migrate into the periblast and occupy a circle which is later characterized by accessory cleavage. This is illustrated in Chart I. A central transverse section of the same egg is shown in Fig. 2.

Chart II.

Chart II A represents the surface view of an egg in the four-celled stage. It was taken from the oviduct at 2 a.m., or about six hours after fertilization. The blastodisc is incompletely divided into four blastomeres which are continuous at their outer margins. They are also continuous below with the yolk, see Chart II C and Fig. 4.

The blastodisc is surrounded by the periblast, the peripheral limit of which as it appears in surface view, is indicated here by a dotted circle. At the inner margin of the periblast there is an incomplete zone of accessory cleavage. Where the accessory cleavage does not appear, the blastomeres are continuous peripherally with the periblast. This fact will be noted again in the description of later stages. (Chart III A, Figs. 9, and 10 A and B.)

In the sections fewer sperm nuclei were found than in the maturation stage, which indicates that a varying number enter the egg (Harper '04, p. 362). A number of sperm nuclei had migrated in superficial positions to the outer margin of the zone which the periblast presents in surface view and others were in the central periblast. Although the central periblast is not at this stage separated from the blastomeres, it may be identified with the finely granular region below the blastodisc, and the thickness of the latter

is determined by the depth of the vertical cleavage plane. The latter measures 0.12 mm. in this egg, and the first horizontal cleavage (Fig. 5) which marks the position of the future segmentation cavity occurs later at the same depth. Compare Figs. 2, 3, 4, 5, 6, and 11,

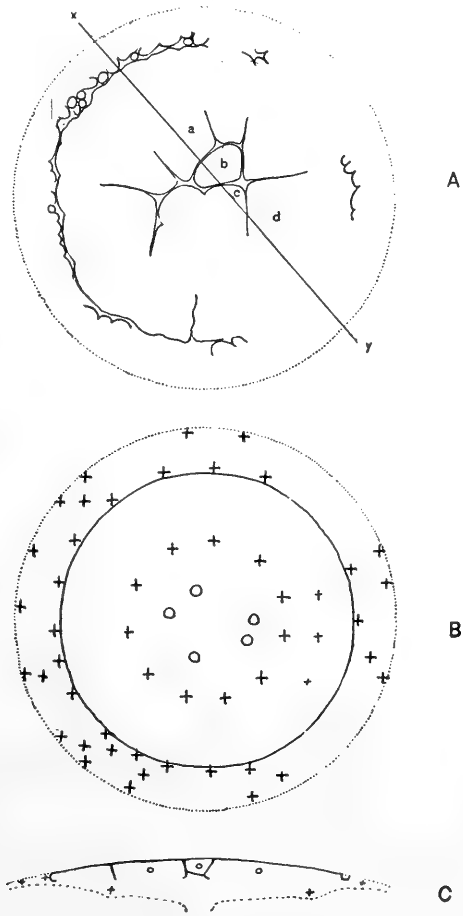


Chart III.

which are drawn to the same scale from central transverse sections of different eggs.

Five primary cleavage nuclei were found in this egg, Chart II B, the nucleus of the cell at the left having divided without a cleavage plane being yet formed.

Chart III.

The next stage illustrated in the charts is that of eight (or perhaps nine) cells, Chart III. The egg was taken from the shell gland at 4.45 a.m., about eight and three-fourths hours after fertilization. The area of primary cleavage presents seven (or eight) large marginal cells, which are continuous with each other at their outer borders, and a smaller central cell which is limited on all sides superficially. This cleavage pattern presents the beginning of the differentiation of the primary area into central and marginal regions, as is more completely illustrated in the sixteen-celled stage (see Fig. 48). The accessory cleavage forms an incomplete circle at the inner margin of the periblast (Chart III A). The marginal cells at the left and posterior side of the blastodisc are definitely limited peripherally by a line just inside of a zone of accessory cleavage. At the anterior and right sides there are three gaps in the accessory cleavage, and no peripheral limit to the marginal cells. In sections, sperm nuclei are not found in this region. This condition of open marginal cells will be referred to in the discussion of later stages, and particularly in the discussion of the periblast. The sperm nuclei have increased in number as compared with the four-celled stage. Compare Charts II B and III B. They occupy superficial positions in the marginal periblast and many of them have migrated to the peripheral edge of the zone which is recognized as the periblast in surface view. They have also migrated deeper into the central periblast and form a submarginal circle.

Chart III C is a diagram of a central transverse section which is shown in detail in Fig. 5. It is taken along the line X Y (Chart III A) and the blastomeres are indicated by corresponding letters in the two figures.

Chart IV.

The next chart (IV) presents the last stage in the multiplication of the sperm nuclei in an egg of about thirty-two cells. The surface view (A) is incompletely represented. The blastomeres seemed to show shrinkage in the salt solution and the egg was hurried on into the killing fluid without taking time to complete the drawing. Enough

of the cells of the central area are drawn, however, to show their size in relation to the marginal cells, and the accessory cleavage was just

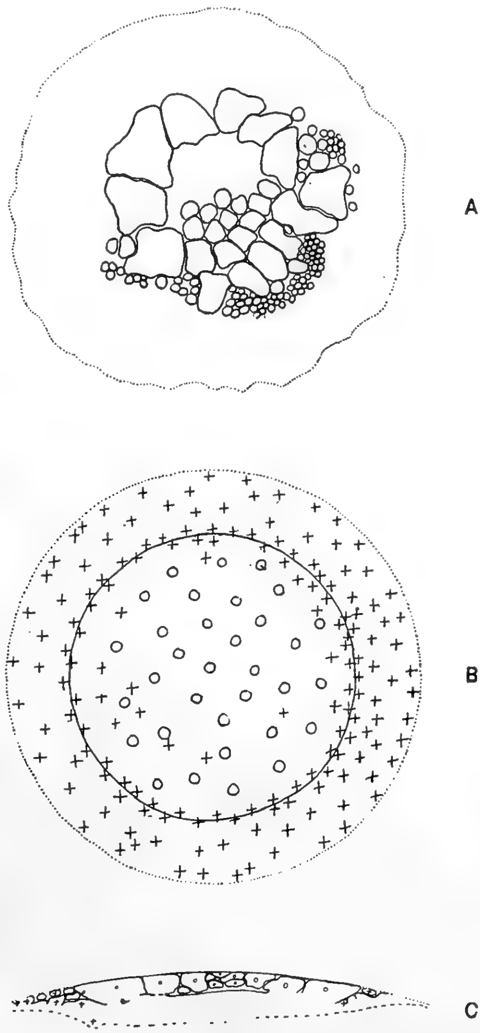


Chart IV.

as abundant on all sides as is represented in the part of the figure completed. The small cells of accessory cleavage were lying one above another in two or in some places three layers (Fig 6).

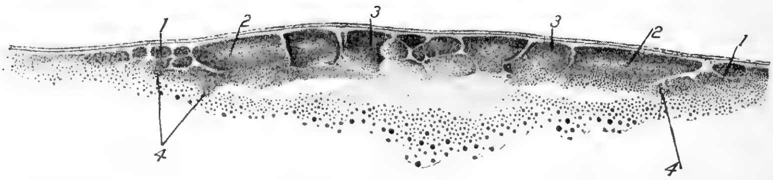


FIG. 6.—Transverse section of a pigeon's egg at the end of the period of multiplication of the sperm nuclei. Egg taken 6:30 a. m., about 10 hours after fertilization and 31 hours before laying. Note that all cells are still continuous with the yolk. 1. Accessory cleavage around the sperm nuclei. 2. Marginal cells sharply separated from the sperm nuclei. 3. Central cells. 4. Sperm nuclei.

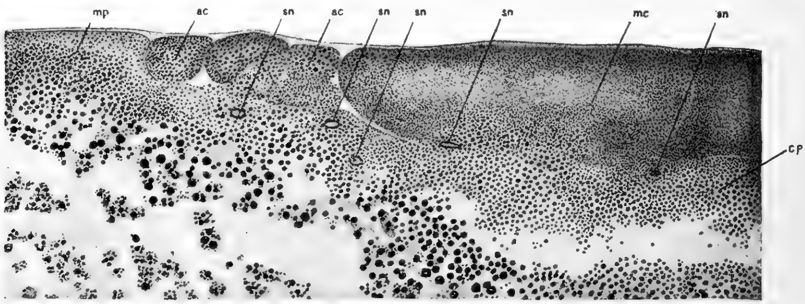


FIG. 7.—The left side of the section shown in Fig. 6. ac, accessory cleavage. cp, central periblast. mp, marginal periblast. mc, marginal cell. sn, sperm nucleus. Leitz, 5/4. Tube length, 140 mm.

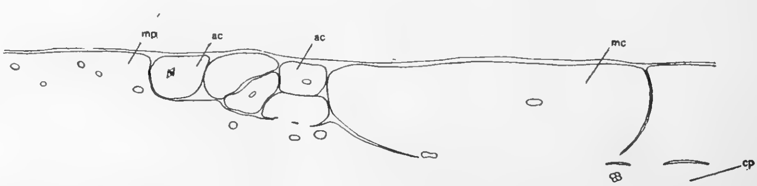


FIG. 8.—Diagram of Fig. 7. The nuclei are reconstructed from eight successive sections. The nuclei in the accessory cleavage and in the marginal and central periblast are derived from supernumerary sperm nuclei. ac, accessory cleavage. cp, central periblast. mc, marginal cell. mp, marginal periblast.

No attempt was made to count the supernumerary sperm nuclei in this egg. They were very abundant, so that in almost any section of the blastoderm several were found in both the marginal and central periblast. They had evidently multiplied by repeated divisions. In earlier stages, the cells of the primary area were separated from the sperm nuclei merely by the cleavage planes enclosing the small accessory cells (see Fig. 5). But in this stage, when the sperm nuclei have become so numerous, the cytoplasm of the blastodisc is definitely separated from the periblast, *i. e.,* there are diagonal or submarginal cleavage planes ventral to the marginal cells of the primary area. See Chart IV C and Figs. 6, 7, and 8. The subgerminal supernumerary nuclei are found as far centrally as the margins of the nucleus of Pander.

Chart V.

Chart V, Fig. A, presents an incomplete drawing of the surface of an egg taken from the oviduct at 7 a.m.—about eleven hours after fertilization. There is no accessory cleavage. The marginal cells are all open peripherally, and only a few of the central cells are drawn to show their relative size. This chart presents a striking contrast to all those that have preceded; for in this egg there was not a single supernumerary sperm nucleus. The nuclei that were found in the sections were all in the cells of primary cleavage with no nuclei in either the marginal or central periblast. There is not usually such an abrupt change as that indicated between the Charts IV and V. Sections of other eggs of about this stage show a diminishing number of sperm nuclei. They do not usually disappear simultaneously on all sides of the blastoderm as seems to have happened in this egg (Chart V). And I have not been able to discover that there is any particular side where they regularly persist longest. Of course, their original position in the egg is variable. But wherever the sperm nuclei do persist, the marginal cells are limited peripherally and ventrally in such a manner as is shown in Chart IV A and C, and Figs. 6, 7, 8, and 10 D to H. And where the sperm nuclei have disappeared, the marginal cells are open peripherally and ventrally, as in Chart V A and C, and Fig. 10 A and B.

Chart VI.

The egg illustrated in Chart VI was taken from the oviduct at 10.30 a. m., fourteen and one-half hours from the estimated time of fertilization. Only a few cells of the surface view were drawn to show relative sizes.

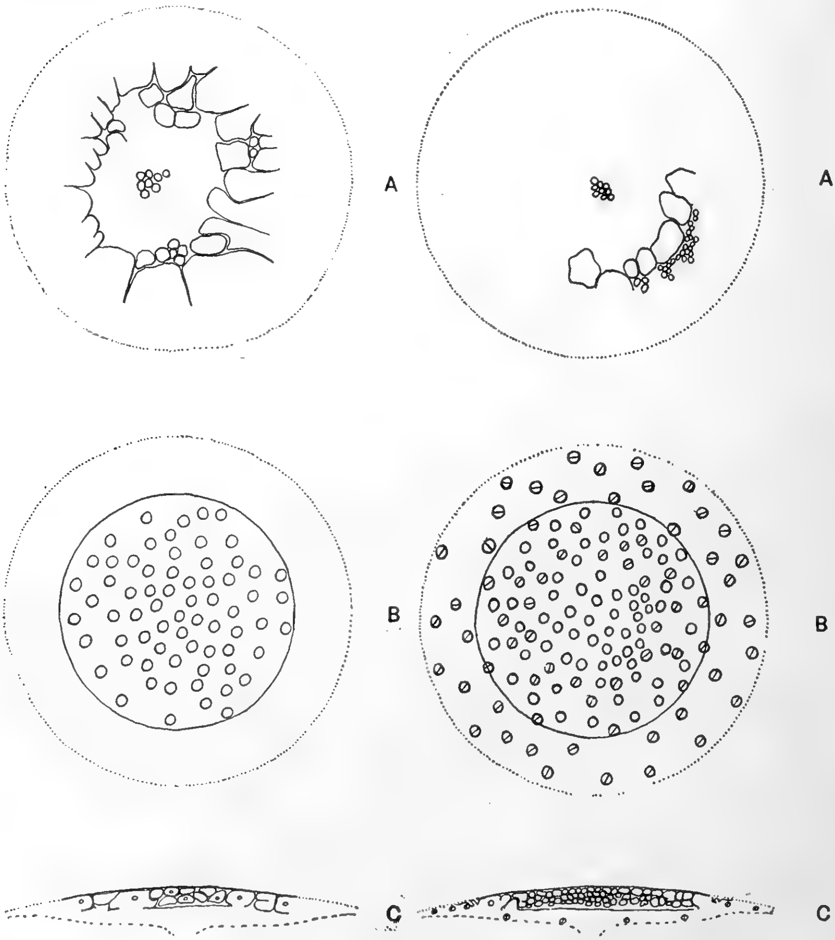


Chart V. Chart VI.

There seems to be a return to accessory cleavage like that shown in Chart IV A. But sections of the egg (Chart VI) demonstrate a relation between the marginal cells and the periblast different

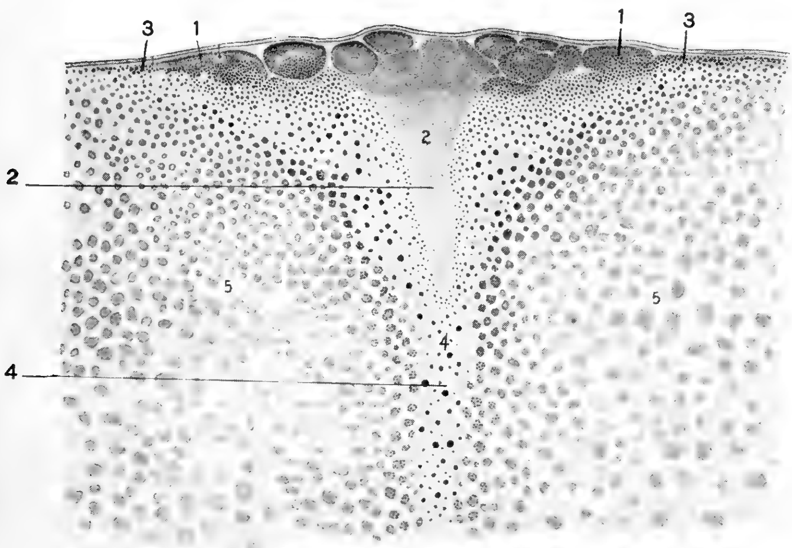
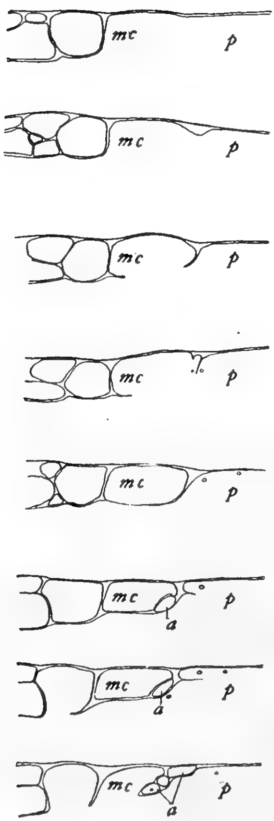


FIG. 9.—Longitudinal section of pigeon's egg at the time of disappearance of the sperm nuclei; on the left (anterior), the marginal cell has become open, *i. e.*, continuous with the marginal periblast. On the right the marginal cell is slightly separated from the periblast at the surface. Surface view of the egg showed traces of accessory cleavage; note continuity of the central cells with central periblast. 1. Marginal cells. 2. Cone of protoplasm. 3. Marginal periblast. 4. Neck of latebra (white yolk). 5. Yellow yolk. Egg taken 7 a. m., about 11 hours after fertilization (estimated).



A
B
C
D
E
F
G
H

Fig. 10.—Diagrams of the posterior side of longitudinal sections through a pigeon egg about the time of the disappearance of the sperm nuclei. A longitudinal median section of the same egg is shown in Fig. 9. In 10 A, the marginal cell is continuous with the periblast, as also at the left of Fig. 9. Fig. 10 B is ten sections beyond A, and here an indentation occurs which in the second section beyond (Fig 10 C) deepens into a cleavage furrow. In the next section (Fig. 10 D) there are two sperm nuclei in the accessory cleavage. They are small and stain very faintly. The wall separating one of the sperm nuclei from the marginal cell has begun to disappear. The other figures (Fig. 10 E, F, G and H) show sperm nuclei either in cells of accessory cleavage or in the marginal periblast, and in either case they are separated from the marginal cell. Through all the sections of this egg, the marginal cells are continuous with the periblast except where sperm nuclei occur. All the nuclei in this figure are sperm nuclei.

a, cells of accessory cleavage.

mc, marginal cell.

p, marginal periblast.

from that which exists in earlier stages, in which the supernumerary sperm nuclei are the cause of the accessory cleavage. This is a stage after the disappearance of the sperm nuclei. The marginal cells are *continuous* with the periblast; the nuclei in the periblast are sisters to the nuclei of the marginal cells, *i. e.*, they are derived from the cleavage nucleus; and the small cells resembling accessory cleavage are mere bud-like and evanescent projections from the periblast. In no section of this egg are there found the diagonal, submarginal cleavage planes like those which separate the primary area from the region of sperm nuclei as shown in Chart IV.

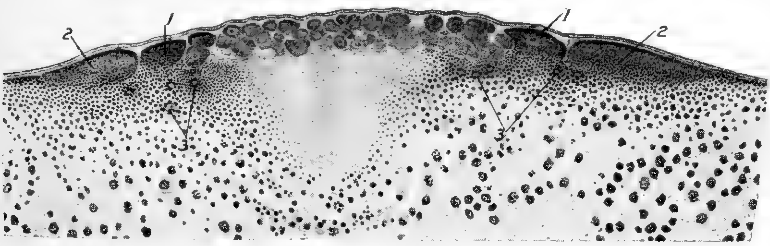


FIG. 11.—Transverse section through the center of the blastoderm of a pigeon's egg taken at 10.30 a. m., 14½ hours after fertilization. 1. Marginal cell. 2. Marginal periblast. 3. Nuclei in the central periblast, derived from the nucleus of the marginal cell.

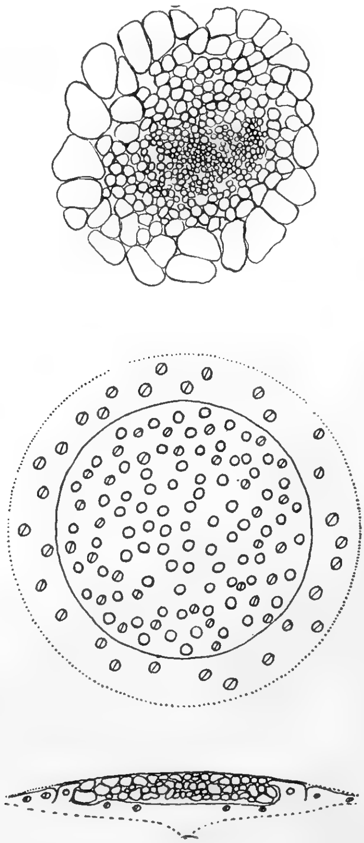
CHART VII.

An egg taken from the bird at 10.30 a.m., fourteen and one-half hours from the estimated time of fertilization, furnishes the data for Chart VII. Although obtained at the same hour as the egg for Chart VI, it was probably fertilized earlier; for it shows a later stage of development. Chart VII A is a free hand drawing, but a photograph of another egg of about the same stage is shown in Fig. 54. At this stage the peripheral limit of the periblast does not show in surface view. In sections of the egg, however, nuclei are found in marginal and central periblast—see Chart VII B and C, and also Fig. 11, which represents a central transverse section of the egg of this chart. From this figure it is plain that the marginal cells constitute a "zone of junction" between the blastodisc and the periblast Agassiz and Whitman, '84).

Summary of the Facts Illustrated by the Charts.

1. During the maturation stage, the supernumerary sperm nuclei migrate from the blastodisc into the periblast.

2. The supernumerary sperm nuclei multiply by division in the periblast and migrate peripherally in the marginal periblast. In sub-



A

B

C

Chart VII.

germinal positions they are found as far centrally as the margins of the nucleus of Pander.

3. As the supernumerary sperm nuclei increase in number, the cells of the primary cleavage are definitely separated from the region of the sperm nuclei by cleavage planes that limit the marginal cells peripherally and ventrally.

4. Between ten and twelve hours after fertilization, the sperm nuclei disappear. This does not occur at any fixed stage of development, but is soon before or soon after the thirty-two celled stage. Certain figures and photographs in this thesis show eggs with fewer than thirty-two cells in which the sperm nuclei are lacking from some sides of the blastoderm, and yet in an egg of thirty-two cells (shown in Chart IV) they are still numerous.

5. After the disappearance of the sperm nuclei, the marginal cells become open peripherally and ventrally, and continuous with the yolk. Nuclei from the marginal cells pass into the periblast and the latter is, therefore, organized with nuclei derived from the cleavage nucleus, and is exactly comparable with the periblast of the bony fish, as described by Agassiz and Whitman ('84).

III. Polyspermy.

Among those plants and animals whose fertilization has been studied, the condition of monospermy is by far the more frequent, so that the idea became prevalent that polyspermy was possible only under pathological conditions. And many investigators have endeavored to solve the problem of why a spermatozoon, and usually only one, enters an egg. It is quite possible that monospermy is secured by different means in different species. But the most satisfactory and most widely applicable explanation of this phenomenon is that a physiological change takes place in the cytoplasm of the egg immediately after the entrance of one spermatozoon, so that conditions are not favorable for other spermatozoa. But polyspermy as a normal condition has been described in a number of animals and in at least one species of plants. Only one sperm nucleus, however, unites with the egg nucleus, and there is no conclusive evidence that the supernumerary nuclei enter into the structure of the embryo in any form, or take part in the formation of the germ layers.

Polyspermy in Bryozoa.—Last year an interesting paper was published by Dr. Kristine Bonnevie on "Physiologische Polyspermie bei Bryozoen." Dr. Bonnevie ('07) found compound spermatozoa, or "Spermazeugmen." She argues that these compound structures are not necessary to secure the power of locomotion for the sperms,

and neither do they facilitate the entrance of the spermatozoon into the egg. Therefore, since the "Spermazeugmen" are not necessary to secure fertilization itself, their function must be to insure polyspermy. "Wir sind so darauf hingewiesen, in der Weiterentwickelung des Eies die Bedeutung dieser eigentümlichen Anpassung zu suchen" (p. 589). She finds in the cytoplasm of the young oocytes of Membranipora pilosa a great quantity of granules and rods containing chromatin. But with the further development of the oocyte, the chromidial apparatus disappears. Finally, with fertilization, the supernumerary spermatozoa provide the somatic chromatin in order to restore the proper nucleo-plasmar relations in the cell.

"Bei der polyspermen Befruchtung wird eine normale Kernplasmarelation wiederhergestellt, indem hierdurch der Oocyte ein neuer Chromidialapparat zugeführt wird. Jedes Spermium enthält ja nämlich in seinem winzig kleinen Kopf eine ähnliche Chromatinmenge wie diejenige, die vor der Befruchtung in der grossen Oocyte vorhanden war. Und während nun, allem Anschein nach, ein Spermakern den männlichen Vorkern liefert, sind die übrigen als die Träger des für den Stoffwechsel der Zelle nötigen *somatischen Chromatins* zu betrachten und haben als solche, auch wenn sie sich nicht zu Kernen entwickeln, eine wichtige Rolle auszuführen.

"Die physiologische Polyspermie bei Membranipora (möglicherweise auch bei anderen Formen) würde nach der hier vertretenen Auffassung nicht als ein isoliertes Phänomen dastehen, sondern sie wäre zwischen den vielen verschiedenen Anpassungen einzuordnen, die in einer günstigen Ausbildung der somatischen Teile des Eies ihr Ziel zu haben scheinen.

"In den allgemein bekannten Fällen solcher Anpassungen werden eine Anzahl junger Eizellen zu Gunsten einer ihrer Schwesterzellen, der heranwachsenden Oocyte, geopfert, entweder indem sie als Nährzellen in dieselbe aufgenommen werden, oder indem sie, wie bei Dytiscus (Giardina, 1901,), einen Teil ihres Chromatins zu derselben abgeben. In unserem Fall, bei der physiologischen Polyspermie, wird die harmonische Weiterentwickelung des befruchteten Eies in ganz ähnlicher Weise gesichert, nur geschieht es diesmal auf Kosten von Zellen des anderen Geschlechtes." (Bonnevie, '07, p. 592.)

Polyspermy in Holothuroidea.—Korschelt and Heider ('03) report a publication by Iwanzoff ('98) on polyspermy in the Holothuroidea. He found that the egg sends out radiating protoplasmic processes through the canals of the zona radiata. The swarming spermatozoa are caught in these processes and are drawn into the egg plasm, and assimilated by it, as Iwanzoff thinks. "Das Ei frisst und verdaut die Spermatozoen." But Iwanzoff observes that when the egg has lost a part of its nuclear substance, in the polar bodies "die Spermatozoen können nicht mehr von der Eizelle bewältigt werden," and only one sperm nucleus can unite with the egg nucleus in fertilization. Here is one more attempt at a teleological explanation of the maturation process.

Polyspermy in Insects.—Among the insects, Henking ('92) found polyspermy in certain of the Hemiptera, Coleoptera, and Hymenoptera. In the egg there is a varying number of micropyles (4 to 7). Each one permits the entrance of a spermatozoon. After fertilization, the spermatozoa are found in the marginal protoplasm, where they degenerate, but they are also found in the yolk where the sperm head changes over into a nucleus.

Polyspermy in Selachians.—Polyspermy has been described in every class of vertebrates except the mammalia. In the selachians, this phenomenon was discovered in an attempt to determine the origin of the free nuclei in the yolk. For a long time it was supposed that these nuclei were derived from nuclei of the blastomeres of early cleavage, or else that they arose in a still earlier stage by a rapid division of the cleavage nucleus. But further research demonstrated these nuclei in maturation stages before the union of the pronuclei, and thus disproved their descent from the cleavage nucleus. Moreover, sperm heads were found in the egg in transitional stages in their process of change into nuclei, and these nuclei in their early mitotic division have the reduced number of chromosomes.

But while there is now agreement as to the origin of the supernumerary nuclei, their fate is still a question for research.

Rückert ('99) identifies the merocyte nuclei of late cleavage stages with those of early cleavage which are derived from supernumerary spermatozoa. But he finds the merocyte nuclei in cells

which are added to the germinal area. They are in such positions as would make it possible for them to enter into the structure of the embryo. Rückert thinks that the cells containing merocyte nuclei may soon degenerate, or if they play any part in the development, it must be only subordinate. (Rückert '99, p. 677). Upon general principle, he is unwilling to admit that nuclei derived from supernumerary spermatozoa enter into the structure of the embryo, and so he finally leaves it an open question whether the merocytes of late cleavage degenerate in the germ layers, or whether their nuclei are not genetically related to merocyte nuclei of early cleavage but are a new set derived from the cleavage nucleus (Rückert, '99, p. 677).

Rückert's Explanation for the migration of the supernumerary sperms.—Rückert presents a theory for the cause of the migration of the supernumerary sperm nuclei. He considers that the sperm nucleus entering first, or the one lying nearest to the female pronucleus, becomes the male pronucleus. With the copulation of the pronuclei, the centrosomes and asters of the male nucleus pass over to the cleavage nucleus. The supernumerary sperm nuclei are repelled from the cleavage nucleus and from each other by the influence of their astral rays. These fibers reach out from the nuclei and when they touch each other, they respond to a stimulus somewhat like thigmotaxis—but in this case, the fibers touch others like themselves instead of a firmer object. When the fibers thus come in contact, the nuclei recoil. The cleavage nucleus being better endowed, drives the other nuclei away from it. The succeeding generations of cleavage nuclei are armed with the protective apparatus ("Schutzvorrichtung"), their inheritance from the male pronucleus, and as cleavage advances, there is therefore a progressive combat between the cleavage nuclei and the supernumerary sperm nuclei for the possession of the germinal area. The cleavage nuclei are stronger and therefore triumph over the weaker, which are continually "driven to the wall." This behavior, Rückert thinks, accounts not only for the expulsion of the supernumerary nuclei (merocyte nuclei) from the central area, but it explains the somewhat equal spacing of them around the periphery and in the deeper parts.

Polyspermy in the Newt.—In the egg of the newt, supernumerary sperms degenerate early. Jordan ('93) found them in the four-celled stage, but not later.

Polyspermy in Reptiles.—Oppel ('92) found the reptile agreeing with the Selachian in respect to the sperm origin of the supernumerary nuclei, but in the Selachian they lead to the merocytes, and in the reptile after a few divisions they remain rudimentary (Oppel, '92, p. 282). Oppel agrees with Rückert that on general principles he cannot believe that the "Nebenspermakerne" enter into the structure of the future embryo (Oppel, '92, p. 283).

In the Kreuzotter (Pelias berus Merr) Ballowitz ('03) found "Nebenspermiumkerne" in the maturation stages. He believes that they give rise to the "Paraspermiumkerne" of later cleavage, and these correspond to Rückert's merocyte nuclei. But Ballowitz says that Rückert's merocytes are derived from sperm nuclei, and that he regards as periblast nuclei only those in the marginal cells remaining in contact with the yolk. Ballowitz, on the other hand, derives the periblast nuclei from daughter nuclei remaining in the yolk and derived from blastomeres. He designates them as "oogenetisch." But he thinks it possible that one of the deep parasperm nuclei may turn back to the floor of the cleavage cavity and remain there as a "spermogenetischen Periblastkern" (Ballowitz, '03, p. 84). The usual origin of the periblast nuclei is the cleavage nuclei. He thinks the parasperm nuclei play no considerable rôle in the development of the germ. But if they come into the coarse yolk under better conditions of nourishment, they may divide and in certain circumstances be added to the germ layers.

Polsypermy in Birds.—Harper ('04) established the fact of polyspermy in the pigeon, but did not determine the fate of the supernumerary sperm nuclei. It has been shown in an earlier part of this paper (p. 13) that in the pigeon's egg, the supernumerary sperm nuclei disappear about ten or twelve hours after fertilization, before or soon after the 32-celled stage. In maturation stages they migrate out of the blastodisc into the periblast, and the longer they remain there, the more definitely they become separated from the blastodisc by planes of cleavage. This fact suggests that they do not enter

into the formation of the germ layers. But they disappear in such an early stage of cleavage, that their participation in the structure of the embryo is out of the question.

Cause of Migration of Supernumerary Sperm Nuclei in the Pigeon. The conditions in the pigeon's egg are not well explained by Rückert's theory of expulsion. Harper ('04) suggests that the "sperm nuclei migrate so early to the periphery of the germinal disc, that it is difficult to believe that they do this under the influence of the cleavage nuclei." I have shown in Chart I and Figs. 2 and 3 that before the union of the pronuclei the supernumerary sperm nuclei have migrated into the periblast. In speaking further of the early migration of the sperm nuclei, Harper says, "This seems to point to the independent activity of the sperm nuclei rather than to any mechanical driving of them from the inner region. What chemotactic influences there may be present, we, of course, have no means of knowing" (p. 378). In another place (p. 372), Harper says: "As an active cause for the migration of the sperm nuclei it might be assumed that the activity is but the continued expression of the labile nature of the protoplasm, which gives the sperm its motile character during the period of its independent existence." I am willing to accept for the pigeon Rückert's theory for the cause of polyspermy in the selachian, namely, the want of protection against it. Harper speaks of the "thinness of the egg membrane when it leaves the tough ovarian capsule." It is commonly accepted among biologists that the egg exerts a chemotactic influence on spermatozoa. Monospermy is secured in many eggs by the cessation of the attractive influence the moment that a spermatozoon enters the egg, *i. e.,* the cytoplasm that is fertilized no longer attracts spermatozoa. Wilson ('03, p. 418) found that spermatozoa enter enucleated fragments of the unfertilized nemertine egg, but they do not enter enucleated fragments obtained after fertilization even in the absence of an egg membrane.

I wish to use this theory of the attractive influence of the egg not only to explain the *entrance* of the spermatozoa, but their *migration* into the periblast. A varying number of spermatozoa (Harper found from 12 to 25 in fertilization stages) enter the pigeon's egg in the vicinity of the egg nucleus. The cytoplasm, then, in this

vicinity is fertilized, but we may suppose that the cytoplasm in the peripheral and deeper parts of the germinal area retains its influence and attracts the supernumerary sperm nuclei into itself just as the cytoplasm of the unfertilized egg attracted the spermatozoa at the time of fertilization.

All of the reported cases of polyspermy in animals are for eggs containing a large amount of yolk, or else those whose peculiar form places much of their cytoplasm at some distance from the egg nucleus. "The union of the germ-cells calls forth profound changes in both" (Wilson, '00, p. 200). Many monospermic eggs are so small that the profound physiological change caused by the entrance of a spermatozoon is almost immediately effective in every part of the egg. Or, indeed, the immediateness of the effect of fertilization may be due in some cases to the character of the protoplasm rather than to the small size of the egg. The germinal area of the pigeon's egg in a state of maturation presents a diameter of about 3 mm. on the surface, and is 0.25 mm. deep in the central part. (These measurements are from sections of the egg shown in Chart I and Figs. 2 and 3). Considering the size, and expanded form of the germinal area, and possibly also some peculiarity inherent in the protoplasm of the pigeon's egg, one may suppose that the peripheral and deeper parts of the cytoplasm retain their attractive power for an appreciable time after the entrance of the spermatozoa into the central superficial region of the germinal area.

If we may then accept these two hypotheses: (1) of the attractive influence of unfertilized cytoplasm upon spermatozoa, and (2) the temporary retention of that influence in parts of the pigeon egg distant from the egg nucleus after the entrance of the spermatozoa; then we may conclude: (1) that the supernumerary sperm nuclei migrate because of the attraction of the cytoplasm on them, and (2) the *path of migration of each nucleus is the resultant of the attractive forces acting upon it.* These two conclusions will, I think, explain the position of the supernumerary sperm nuclei in the pigeon and selachian.

The spermatozoa enter the egg in the vicinity of the egg nucleus. Since their number varies, their distribution also varies in different

eggs. But each nucleus is attracted by the entire mass of cytoplasm outside of the central fertilized area. Since the germinal area of the pigeon's egg is shallow, the attractive force towards the central, deeper part is less than that towards the periphery, and, therefore, the sperm nuclei are drawn out to the periphery, *i. e.,* into the marginal periblast. But each nucleus, as it goes, leaves behind it a fertilized path. This path, or radius, is then eliminated from the attractive influence on any other nucleus. Another nucleus in its original position near the first one referred to would pass out along another radius, but since radii diverge toward the circumference, the nuclei would take positions in the periblast further from each other than they were in their original positions near the center. This explains the more or less regular spacing of the nuclei around the periphery. The application of this principle of attraction also explains the position of the supernumerary nuclei in the central periblast. If the cytoplasm near the surface was fertilized by other migrating nuclei, the attractive influence of the remaining cytoplasm would lead these nuclei into positions further below the surface. Of course, I do not mean that the nuclei migrate successively. They pass simultaneously each one along the resultant of the attractive forces which act upon it. As they go, the influence of fertilization emanates in all directions from each nucleus and so eliminates the attraction from all directions, except the line of migration which the nucleus is to follow.

If these hypotheses are applied to the selachian egg, the positions of the merocyte nuclei there are easily explained. The germinal area of the selachian egg is proportionally much deeper than that of the pigeon. It approaches the shape of a hemisphere with the convex side toward the yolk. There is, therefore, a proportionally greater attraction toward the deeper cytoplasm in the selachian egg than in the pigeon egg, and the "merocyte" nuclei are found deep. See Rückert ('99), Figs. 37, 39 and others. Judging from descriptions by Oppel and Ballowitz, I think this explanation will also serve for the conditions in the reptilian egg.

Polyspermy in Plants.—There remains yet to mention the case of polyspermy in plants. Land ('07) found that the second male

nucleus enters the cytoplasm of the egg of Ephedra trifurca. "As it disintegrates, minute cells appear, which are believed to be the joint product of the chromatin of the second male nucleus and the chromatin of some of the jacket cells; these minute cells at least foreshadow the endosperm of angiosperms, and may be called physiological endosperm" (p. 290).

This case of the second male nucleus in the cytoplasm of the egg of Ephedra is exactly parallel with the case of supernumerary sperm nuclei in the cytoplasm of the pigeon's egg. The case of "double fertilization" or "triple fusion" in the Angiosperms which is often compared with polyspermy in animals, is not a parallel case; for in the "triple fusion" the second male nucleus unites with two polar nuclei, one of which is *sister to the egg*. From this fusion the endosperm is formed which is believed to nourish the embryo. Henneguy ('02) described the entrance of spermatozoa into the yolk cells of Distomum hepaticum, and he regarded the yolk cells as abortive egg cells. These are offered for the nourishment of the egg and the condition is quite parallel to the "triple fusion" of Angiosperms.

Accessory Cleavage and Segmentation of Egg Fragments.—The division of the supernumerary sperm nuclei and the "accessory cleavage" present a problem of interest. In spermatogenesis, there is no division of the nucleus after the formation of the spermatid. So far as we know, the spermatozoa do not divide in any medium in which they are normally found, except in the cytoplasm of the egg. But in the case of physiological polyspermy, the spermatozoa are in a medium where any of them that becomes the successful male pronucleus will divide after union with the female pronucleus. Therefore, the supernumerary sperm nuclei in the egg cytoplasm may divide. The accessory cleavage of the selachian's and bird's egg is certainly comparable to the division of fertilized enucleated fragments of the sea urchin's egg or nemertian egg.

"Inwandering Follicular Cells."—Harper ('04) illustrates an "inwandering follicular cell." (Harper, '04, Pl. II, Fig. 7 i). I have found a large number of cells in the perivitelline fluid in the egg represented in Chart II. Harper's figure does not show the

environment of the structure, and I cannot be sure that it is homologous with those illustrated in Figs. 12a and b, and 13. I think the latter are supernumerary sperms. Rückert ('99, p. 671) discusses the question of inwandering female elements, and while he thinks there is no absolute proof against such a phenomenon, he has nothing to present in its favor.

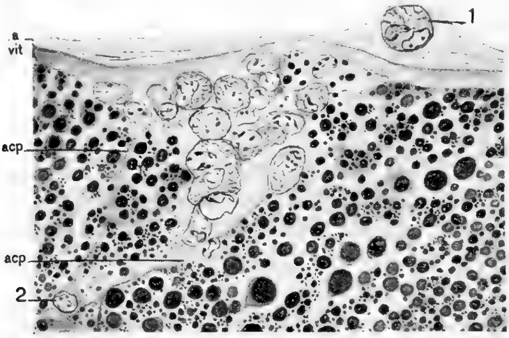


Fig. 12a.—A mass of sperm nuclei in the region of accessory cleavage in the egg shown in Chart II A. Transverse section. The center of the blastoderm is toward the right. a., thin layers of albumen adhering to the egg. acp., planes of accessory cleavage. vit. vitelline membrane. 1. Spermatozoen caught between two layers of albumen. 2. A sperm nucleus in the deepest part of the plane of accessory cleavage. Leitz $4 / \frac{1}{12}$. Tube length 140 mm.

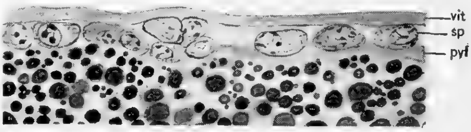


Fig. 12b.—From the same section as Fig. 12a. From the marginal periblast to the left of Fig. 12a. vit., vitelline membrane. pvf., perivitelline fluid. sp., sperm nucleus. Leitz $4 / \frac{1}{12}$. Tube length 140 mm.

Fig. 12a is taken from a transverse section of the egg shown in Fig. 4 and Chart II. It is in the accessory cleavage, the right hand side of the figure being toward the center of the blastoderm. Fig. 12b is from the same section, but further peripheral in the periblast. There were other cells between these two places. This particular group of nuclei continued through ten sections (each section 10 microns) with similar conditions in other parts of the

same egg, but I have not found such a mass of them in any other egg. The nuclei are in the perivitelline fluid and at the place shown in Fig. 12a they have collected in the furrows of accessory cleavage, and one of them (2) has followed a furrow to its deepest limit. In this furrow some of the nuclei seem to be in a syncytium without definite cell boundaries, while others are in entire and separate cells. One such cell is enclosed between two delicate layers of egg albumen (Fig. 12a, 1). Its nucleus has divided—perhaps amitotically.

There are two suggestions for the origin of these peculiar cells. They may be:

1. inwandering follicular cells, or
2. supernumerary spermatozoa.

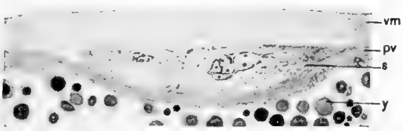


FIG. 13. —From a longitudinal section of a pigeon's egg, eleven and three-fourths hours after fertilization, 7.15 a. m. vm., vitelline membrane. pv., perivitelline fluid. s, one of the supernumerary spermatozoa. y, yolk. Leitz $4 / \frac{1}{12}$ Tube length 140 mm.

If they are follicular cells, we should expect to find them occurring quite constantly in all of the eggs of the pigeon. These structures occur in 33⅓ per cent of the eggs that I have studied, from four to fifteen hours after fertilization. They occur in only 25 per cent of my pigeon eggs from fifteen to thirty-nine hours after fertilization, and not at all in later stages. Degeneration is their fate. In the stages later than fifteen hours from fertilization, these cells were very scarce,—perhaps only one or two in an egg (Fig. 13). If the follicular cells wander into the egg, there must be some quite regularly occurring cause for their leaving the follicular epithelium. We would not expect them to leave a compact epithelium unless they are by nature wandering cells. Von Brunn ('82) describes the entrance of follicular cells into ovarian eggs that are in the process of resorption, but says in a footnote, p. 6, "Ueberhaupt ist ja wohl

kein Fall bekannt, dass die Wanderkörper ins Innere normaler vegetationsfähiger Zellen eingedrungen wären und dieselben zerstört hätten."

Are these cells supernumerary spermatozoa? Their inconstant appearance argues for this origin. Possibly they are sperms that have entered late. The influence of the egg which otherwise would have drawn them into the granular cytoplasm may have ceased just after they had gone through the vitelline membrane, and they may have migrated peripherally in the perivitelline fluid to the region of the periblast. Or they may have entered late in the region where they are now found. They are usually in the region of the periblast. Their entrance directly into the periblast without migration from the blastodisc would make them comparable with the spermatozoa which enter the vegetative pole of the amphibian egg, or those which Ballowitz ('03) has described as entering directly into the yolk of the adder. Their usual degeneration at about the time when the supernumerary sperm nuclei degenerate in the periblast also argues for their sperm origin.

Moreover, we can hardly believe that so many follicular cells would enter at one place as to give such a large mass as is found in Fig. 12a. Even by repeated division until the time when this egg was killed, six hours after fertilization, two or three follicular cells which might enter together could not form so many as are in this mass.

Evidence of the Disappearance of the Supernumerary Nuclei in the Pigeon and Comparison with Other Meroblastic Vertebrate Eggs.—As I follow the figures and descriptions of the reptilian and selachian eggs, I must believe that in those forms, as in the pigeon, the supernumerary nuclei degenerate early, and that the "yolk nuclei" of later cleavage are *periblast* nuclei derived from the cleavage nucleus. Rückert's ('99) Figs. 2, 3, 4 and 8, Tab. LII, give surface views of successive stages of Torpedo eggs. With the progress of development, the accessory cleavage disappears. In the pigeon's egg, with the disappearance of the accessory cleavage at the surface, there is also the absence of supernumerary nuclei in the sections. But it may be suggested that the egg in which I saw no

accessory cleavage on the surface, and in the sections no nuclei outside of the blastomeres (the egg of Chart V) was monospermic. I cannot prove the contrary in the case of this particular egg. My argument for the disappearance of the supernumerary sperm nuclei, the establishment of a zone of junction and the organization of the periblast with nuclei from the marginal blastomeres does not rest upon this alone. The presence of the well marked line of cleavage separating the blastodisc from the periblast where the sperm nuclei exist and the absence of those cleavage planes from other parts of the same egg where there are no sperm nuclei, and the constant continuity between blastodisc and periblast after about twelve or fourteen hours after fertilization, are facts which argue strongly for the difference in character of the free nuclei in early and late stages.

My argument for the disappearance of the supernumerary sperm nuclei and the subsequent opening of the marginal cells to become continuous with the periblast, is further supported by observations of a living egg through about eight hours of development. I saw the egg first at 2.15 a. m. and made a free hand sketch of the surface view (Fig. 16 A). Fig 16 B is a sketch of the same egg at 2.50 a.m. The accessory cleavage has increased slightly. The cleavage planes have become more distinct. A little later, I saw white spots appearing in the periblast. They probably marked the position of the supernumerary sperm nuclei. I watched one particular spot, and saw it elongate and divide into two. As the supernumerary nuclei multiplied, the peripheral outlines of the marginal cells became more distinct, but where there were no nuclei in the periblast, the marginal cells were continuous with it. At 5.15 a. m. the lines of accessory cleavage had become indistinct, and finally, at 10.00 a. m. (when the egg was killed) there were only faint traces of accessory cleavage, although some white spots remained, indicating the presence of supernumerary nuclei. They disappeared more slowly in this egg, perhaps, than in normal conditions, at any rate there was little change in the egg during the last three hours that it was under observation. (The egg has not yet been sectioned).

I have placed no emphasis on the histological characters of the nuclei, because there is great danger of misinterpretation here.

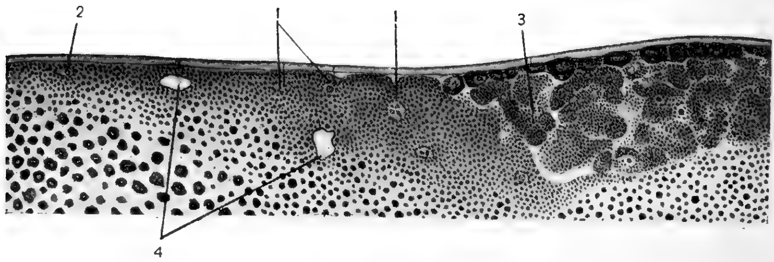


FIG. 14.—Posterior side of a longitudinal section of a pigeon's egg about twenty-five hours after fertilization, 8.50 p. m. 1. Nests of periblast nuclei. 2. Periblast nucleus. 3. Syncytial mass derived from the periblast, organizing into cells which will be added to the blastodisc. 4. Vacuoles.

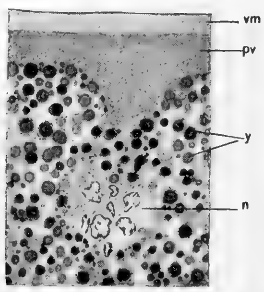


FIG. 14a.—From the section as shown in Fig. 14. vm., vitelline membrane. pv., perivitelline fluid. y, yolk granules in the marginal periblast. n, the nuclear nest numbered 1 and nearest to the cleavage in Fig. 14. Leitz $4/\frac{1}{12}$. Tube length 140 mm.

Harper found eight chromatin vesicles in what he supposed were sperm nuclei in a late cleavage stage. But they were periblast nuclei, and the number of vesicles is not significant. See Figs. 14 and 14a. Rückert finds merocytes among the cells of the germ layers, and admits that it is impossible to distinguish their nuclei from others whose origin is the cleavage nucleus. But if the merosyte nuclei of the selachian are derived from the marginal blastomeres, they naturally do resemble other nuclei in the germ layers. If the reptiles and selachians would breed in confinement, so that the approximate time of fertilization could be determined, and if a close series of stages could be obtained from them as has been done from the pigeon, the material would probably demonstrate the early disappearance of the supernumerary sperm nuclei and the subsequent organization of a periblast homologous with that already described for the teleost (Agassiz and Whitman, '84,) and for the bird (Blount, '07). The homology of the periblast with the vegetative pole of the holoblastic vertebrate eggs will be discussed in another part of this paper (p. 49).

Function of the Supernumerary Sperms.—There still remains the question of the function of the supernumerary sperm nuclei. Bonnevie suggested that they provide the somatic chromatin and that they are offered for "die harmonische Weiterentwickelung des befruchteten Eies." But the variation in the number of spermatozoa does not support this suggestion. In the pigeon, Harper found from 12 to 25 supernumerary sperms. Rückert found in fertilization stages among nineteen areas of Pristiurus from 7 to 47, and in twenty germ areas of Torpedo in the same stages from 1 to 56 supernumerary nuclei. In the stage of the first cleavage nucleus of the adder, Ballowitz found in nine germ areas a variation from 8 to 36 supernumerary nuclei. In one-fifth of all cases, Oppel could not prove the presence of merocyte nuclei. Henking found forty-seven monospermic and forty-eight polyspermic eggs of one of the hemiptera. If one supernumerary spermatozoon is enough to restore the nucleoplasmar balance in the egg of Torpedo, fifty-six are too many. If half of the eggs of one of the hemiptera develop after the entrance of only one spermatozoon, more than one is not

necessary for "die harmonische Weiterentwickelung des befruchteten Eies." And Henking is satisfied that both the monospermic and polyspermic eggs proceed to normal development.

Rückert has suggested that perhaps the merocyte nuclei favor the division of the marginal blastomeres. In the pigeon, the primary cleavage in early stages is far from what we must suppose to be the sphere of influence of the supernumerary nuclei. It is not until after the disappearance of the latter that there is much division of the marginal blastomeres.

A nutritive function has often been assigned to these nuclei, as if they digest the yolk and pass the products on to the developing

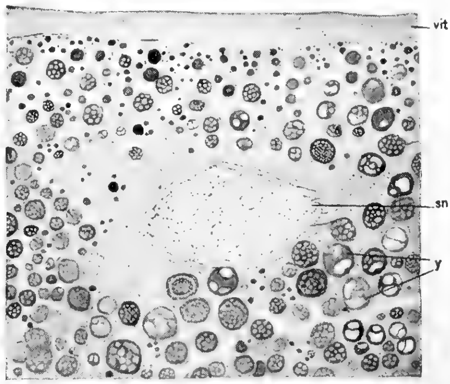


FIG. 15.—A nucleus derived from one of the supernumerary spermatozoa, and its surroundings. This is the more central of the two nuclei at the right-hand side of Fig. 2. sn., sperm nucleus. vit., vitelline membrane, y, yolk granules showing signs of digestion. Leitz $4/\frac{1}{12}$. Tube length 140 mm.

germ. Fig. 15 shows a sperm nucleus in a maturation stage (one of the nuclei in Fig. 2). It seems to be digesting the yolk around it. Rückert insists that even if they do degenerate later, they may be indispensable for nutrition in the early stages. I cannot prove that they do not function in this way. However, since the entrance of the supernumerary spermatozoa into the egg is more or less a matter of chance (in the sense that anything can be chance) and since their number varies, I cannot believe that their presence is essential to normal development. I believe that if but one spermatozoon entered the pigeon's egg, normal development might ensue.

Unfortunately, this cannot be demonstrated experimentally. It seems to me that the entrance of more than one spermatozoon is a casual event. The same condition in the egg that secures fertilization (the attractive influence) also takes care of the supernumerary spermatozoa, be they many or few. I do not consider it a protective device (Schutzvorrichtung).

IV. Area of Primary Cleavage.

Maturation Stage.—The surface view of the pigeon's egg before the appearance of the first cleavage plane has been previously described (p. 4, Chart I A, and Fig. 45). The periblastic zone is not conspicuous in all eggs in the maturation stage. What causes the differentiation between periblast and blastodisc, I cannot explain at present. Possibly the two areas are not distinguished from each other until the migrating sperm nuclei come to rest at the inner margin of the periblast.

Direction of the First Cleavage Plane.—The first cleavage plane may appear about 1 a. m., five hours after fertilization. Its position, and direction in relation to the axis of the future embryo seem not to be constant. There are, of course, occasional variations in the orientation of the embryo. I have found pigeon eggs in which the primitive streak was nearly parallel to the chalazal axis, and others in which the angle with the chalazal axis was about 60°, but the anterior end was toward the left instead of the right (see Fig. 1). These abnormalities in orientation are comparatively rare. But it may be that some of the eggs which I have studied in the two-celled stage which present variations in direction of the first cleavage plane, would have shown an abnormal orientation of the embryo. One egg obtained about 1.15 a. m., 5¼ hours after fertilization, had the first cleavage plane making an angle of about 7° with the chalazal axis. Another egg (Fig. 18) apparently has the first cleavage plane parallel to the longitudinal axis of the embryo, although this may be the second cleavage, and the first may have nearly disappeared.

I watched the development in another egg (Egg 404) (Fig. 17). When I first saw this egg at 3 a. m., there was one cleavage plane

(1+2) parallel to the axis of the embryo, but in an excentric position. In another egg (Egg 420, Fig. 16 A) in which I watched the development, there was one cleavage plane (1+2) visible at 2.15 a. m. and it was transverse to the axis of the future embryo. But these lines are not constantly in view. As the blastomeres of the

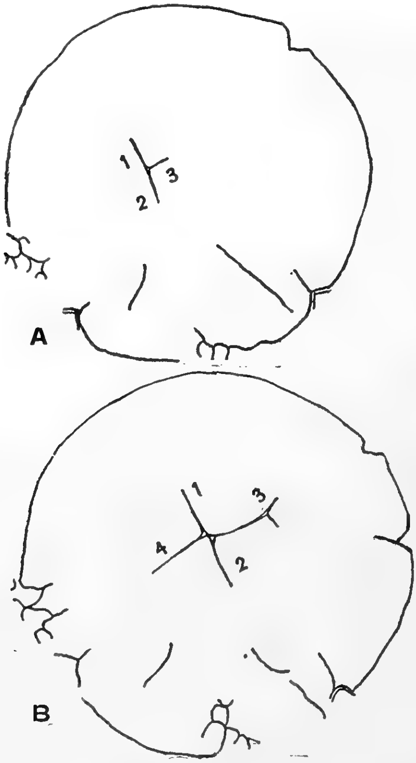


Fig. 16.—Two views of a living pigeon egg. A was drawn at 2.15 a. m., six and one-fourth hours after the estimated time of fertilization. B was drawn about forty-five minutes later, between 2.50 and 3.05 a. m.

living egg separate from each other, the furrow between them is widened and is then easily seen. But when the cells press closely together, the cleavage furrow fades from view. Thus it may be that when I first saw Egg 404 (Fig. 17) the plane 3 + 4 may have been temporarily invisible, but may have been really the first cleavage furrow, and 1+2 the second.

Kölliker ('76, Fig. 16) gives a figure of a hen's egg in the two-celled stage, in which the first cleavage plane is transverse to the longitudinal axis of the embryo. He describes it as follows: "Die Keimscheibe war weiss, nahezu 3 mm. gross, von einem schmalen dunklen Hofe umgeben und durch eine mittlere bogenförmige Furche unvollständig in zwei Hälften geschieden." The "dunklen Hofe" is, I think, the periblast.

The Four-celled Stage.—I present a series of figures (18 to 26) of pigeon eggs in approximately the four-celled stage. The orientation is the same as indicated in Fig. 1. (Compare Fig. 25 with Kölliker ('76) Fig. 17.) In these two figures, the cleavage planes are in exactly the same relation to the chalazal axis, but in the

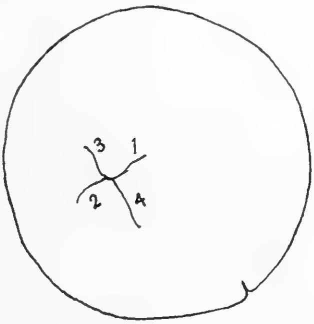


FIG. 17.—A living pigeon egg. When the egg was obtained, the cleavage furrow 1 + 2 was the only one in view. 3 appeared at 3.30 a. m., and 4 came at 3.35 a. m. The egg was obtained about five hours after fertilization.

hen's egg the axis of the embryo is at right angles to the chalaza, and in the pigeon's egg the embryo is diagonal (Fig. 1). A comparison of all these figures suggests that the early cleavage planes of the pigeon's egg bear no constant relation to the axis of the future embryo.

Asymmetry.—These figures also suggest what is confirmed in later stages (see Figs. 27 to 40 and see photographs), that the cleavage is not *always* excentric, as represented by Kölliker, "Die Furchung geht immer asymmetrisch vor sich, so dass ohne Ausnahme die eine Hälfte der Keimscheibe in der Zerklüftung der andern voran ist und die Hauptmasse der Kugeln und ebenso die kleineren Segmente

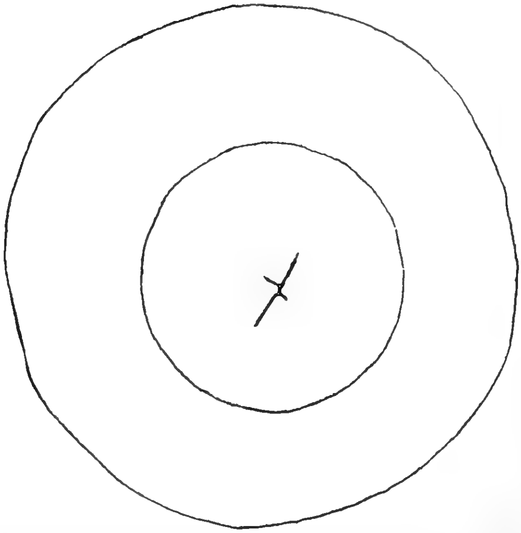


FIG. 18.—Surface view of a pigeon's egg taken from the oviduct at 11.35 p. m., 7 hours and 40 minutes after the bird had laid another egg. The longer cleavage plane is parallel to the axis of the future embryo. There was no accessory cleavage, but the periblast and blastodisc were differentiated.

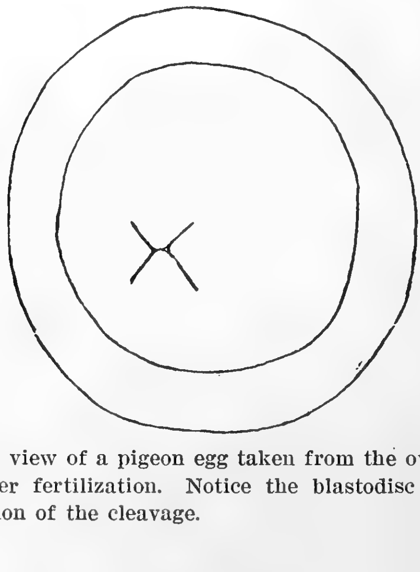


FIG. 19.—Surface view of a pigeon egg taken from the oviduct at 1.00 a. m., about 5 hours after fertilization. Notice the blastodisc and periblast, and the excentric position of the cleavage.

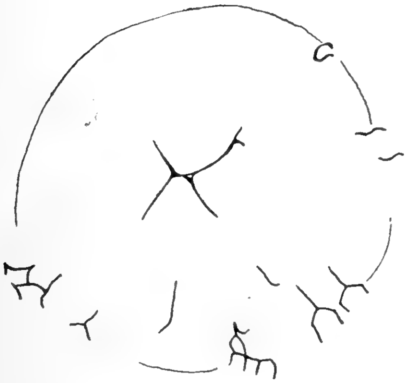


FIG. 20.

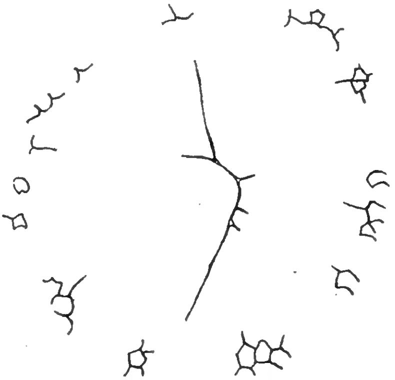


FIG. 21.

FIG. 20.—Surface view of pigeon egg as it appeared at 3.00 a. m., about seven hours after fertilization.

FIG. 21.—Surface view of pigeon egg obtained at 3.30 a. m., seven and one-half hours after the estimated time of fertilization.

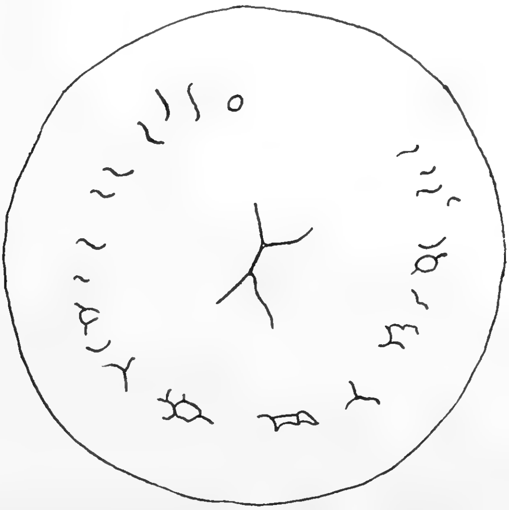


FIG. 22.—Pigeon egg taken at 2.00 a. m., six hours after the estimated time of fertilization. The same egg is shown in Chart II A.

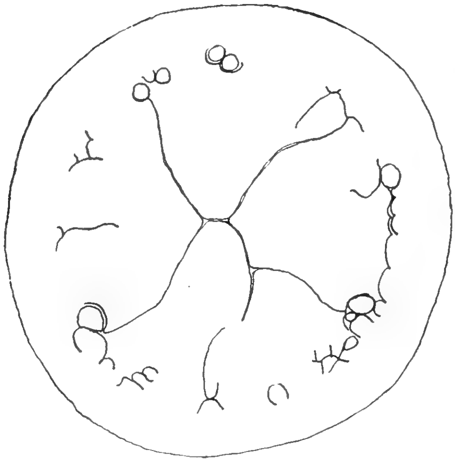


FIG 23.

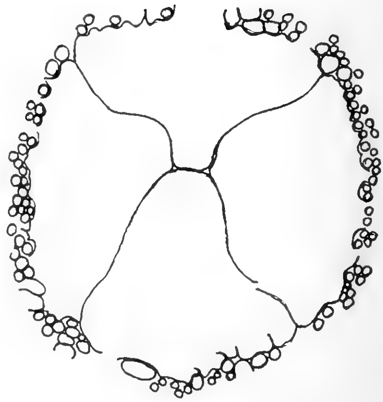


FIG. 24.

FIG. 23.—Pigeon egg obtained at three o'clock a. m., about seven hours after fertilization.

FIG. 24.—Pigeon egg obtained at 4.00 a. m., eight hours after the estimated time of fertilization.

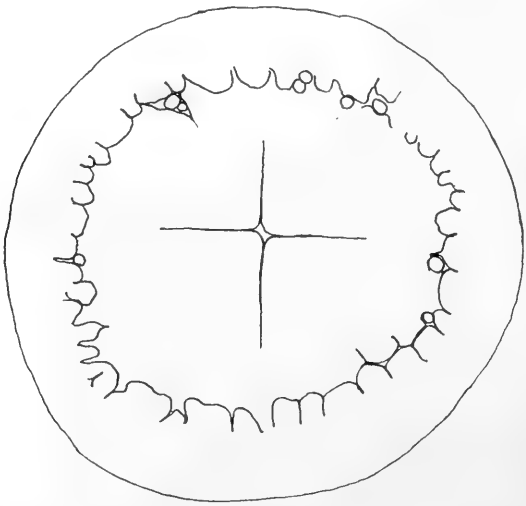


FIG. 25.—Pigeon egg taken from oviduct at 3.15 a. m., about seven and one-fourth hours after fertilization.

und kleineren Kugeln der einen Hälfte der Keimscheibe angehören und der Mittelpunkt des Feldes mit Furchungskugeln excentrisch liegt." (Kölliker ('76), p. 79.)

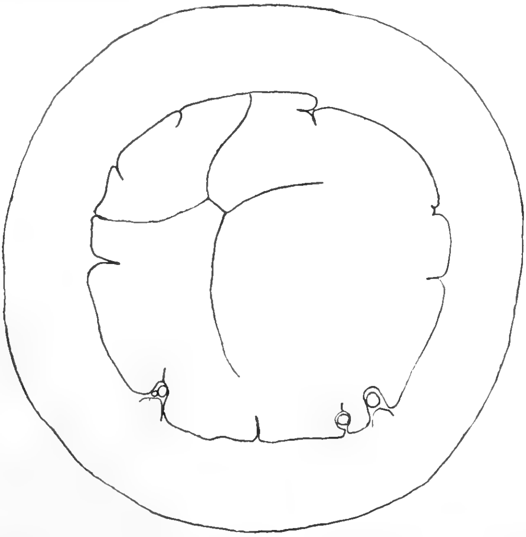


Fig. 26.—Pigeon egg six and one-half hours after the estimated time of fertilization, 2.30 a. m.

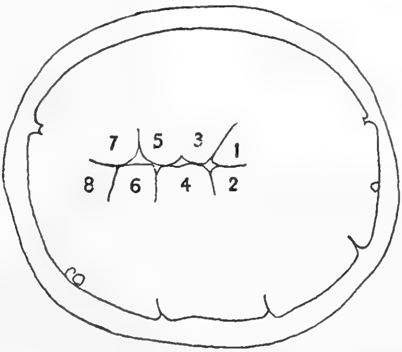


Fig. 27.—Surface view of a pigeon egg at 3.15 a. m., about seven and one-fourth hours after fertilization. Compare with Figs. 28, 29 and 30. The numbers suggest homologies in cells, but have no reference to order of cleavage.

The Eight-celled and Later Stages.—Eggs in approximately the eight-celled stage are shown in Figs. 27 to 37. There is here quite a variety in the cleavage pattern. Fig. 27 lacks only one plane

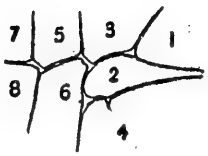


Fig. 28.

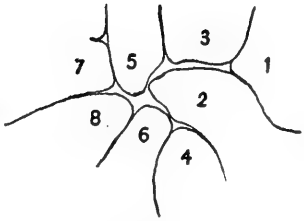


Fig. 29.

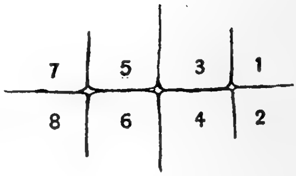


Fig. 30.

Fig. 28.—Pigeon egg at 4.30 a. m., eight and one-half hours after fertilization. Compare Figs. 27, 29 and 30.

Fig. 29.—Pigeon egg at 4.45 a. m., eight and three-fourths hours after fertilization. Compare Figs. 27, 28 and 30.

Fig. 30.—Diagram of the teleost egg in the 8-celled stage. Compare with Figs. 27, 28 and 29.

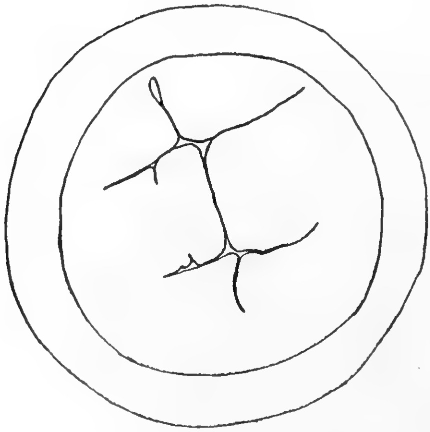


Fig. 31.

Fig. 32.

Fig. 31.—Pigeon egg at 3 a. m., seven hours after fertilization.

Fig. 32.—Pigeon egg obtained at 4.30 a. m., eight and one-half hours from fertilization.

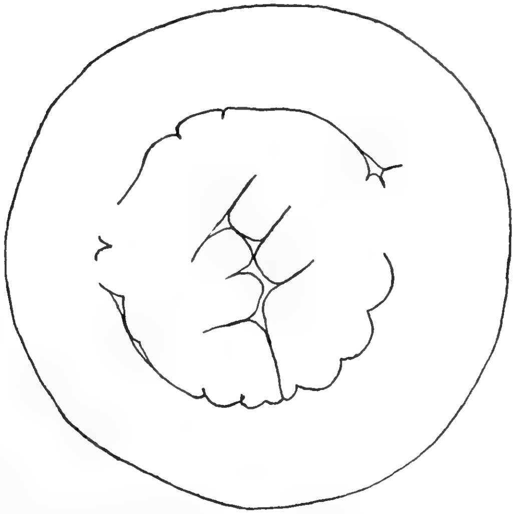


Fig. 33.—Pigeon egg at 4.00 a. m., eight hours after fertilization.

Fig. 34.

Fig. 35.

Fig. 34.—Pigeon egg at 2.30 a. m., six and one-half hours after fertilization.
Fig. 35.—Pigeon egg at five o'clock a. m., nine hours after fertilization.

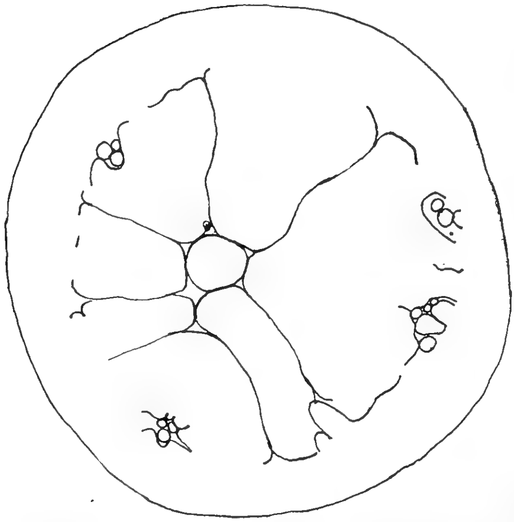


FIG. 36.—Pigeon egg at 4.15 a. m., eight and one-fourth hours after fertilization.

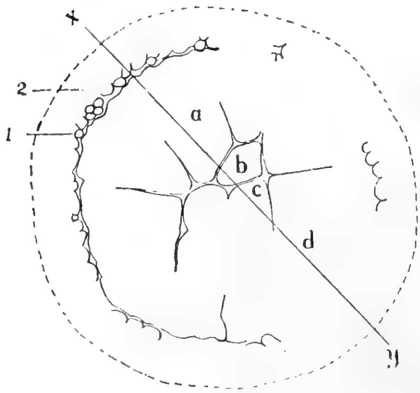


FIG. 37.

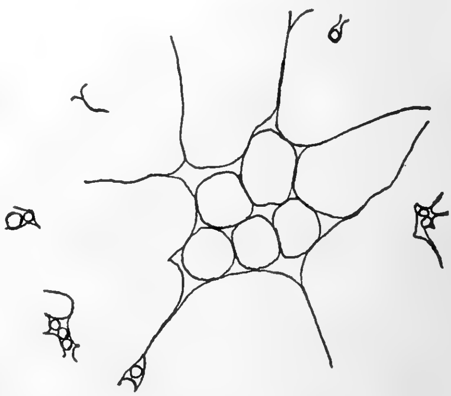


FIG. 38.

FIG. 37.—Pigeon egg at 4.45 a. m., eight and three-fourths hours after the estimated time of fertilization. 1. Accessory cleavage. 2. Periblast. a, b, c, d, cells of the primary cleavage which are indicated by corresponding letters in Fig. 5. The section represented in Fig. 5 is taken along the line xy.

FIG. 38.—Pigeon egg at 5.00 a. m., nine hours after fertilization.

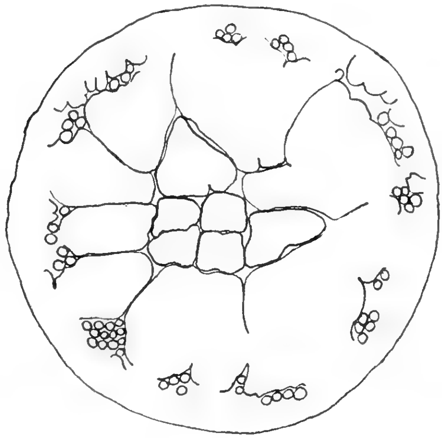


FIG. 39.—Pigeon egg at 5.15 a. m., nine and one-fourth hours from fertilization.

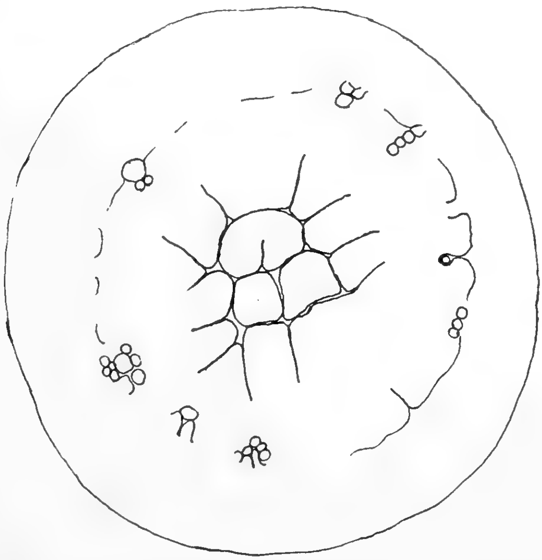


FIG. 40.—Pigeon egg at 4.45 a. m., eight and three-fourths hours after fertilization.

from being identical with Harper's ('04) Fig. 43, and these both resemble the eight-celled stage of the teleost (Wilson, H. V., '91, Fig. 6, and Agassiz and Whitman, '89, Pl. xx, Fig. 19). Fig. 30 is a diagram of the teleost egg in the eight-celled stage. It is numbered for comparison with certain pigeon eggs, Fig. 27, 28, 29. The numbers merely indicate the homologies of the cells and are not intended to signify the order of segmentation.

Following the eight-celled stage, cells are cut off from the central ends of the large blastomeres and there are then established three principal regions, as I have described in my preliminary paper: (1) the central area, (2) the marginal cells, (3) the periblast. The cells of the central area are Kölliker's "Furchungskugeln." The marginal cells are his "Segmenten." As cleavage proceeds, cells are cut off centrally from the marginal cells and added to the central area, and thus the latter grows at the expense of the former. Radial cleavage planes divide the marginal cells, and increase their number while the central cells are constantly becoming smaller by division. This is well illustrated in photographs of a number of different eggs (Figs. 46 to 54) and especially in two photographs of the same egg (358), (Figs. 50 and 51).

Finally, the marginal cells are all used up and we recognize only two regions in the blastoderm: (1) the central area, and (2) the periblast. In early stages, all of the cells are continuous with the yolk (see Fig. 4), but as development proceeds the central cells become complete below and separate from the yolk (see Figs. 5, 6, 11) and only the marginal cells are open below. Thus the marginal cells constitute a "zone of junction" (see Agassiz and Whitman, '84, Figs. 2, 3, 4, and 5) between the segmented and unsegmented parts of the egg.

V. The Segmentation Cavity.

Description by Duval.—The position of the segmentation cavity in the bird's egg has been a subject of considerable discussion. Duval ('84) gives two figures (Figs. 4 and 5) of longitudinal sections of just laid, unfertilized hen's eggs, in which he identifies the segmentation cavity as a small space just below the upper layer

of cells. Below the segmentation cavity there is a layer of cells continuous with the yolk. Stages of later cleavage of the pheasant and the canary are shown in Duval's Figs. 7 and 8 respectively. Here again the segmentation cavity is below the upper layer of cells, *i. e.*, it is between the upper layer and a mass of cells which has been added to the germinal area from the syncytial yolk.

Description by Kölliker.—Kölliker ('76) describes the blastoderm of the hen's egg as increasing in depth by additions from the "Bildungsdotter." His Fig. 18 presents the surface view of the germ area of a hen's egg in which there are twenty-one cells, and his Fig. 19 gives a vertical section through the same egg. This section shows one layer of four cells in which there are two central "Furchungskugeln" and the two marginal "Segmenten." Below this layer of cells is the unsegmented "Bildungsdotter" and below that is the funnel-shaped white yolk (Fig. 41). He describes the blastoderm as increasing in depth as cleavage progresses. None of the

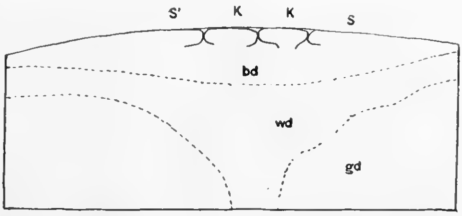


FIG. 41.—Copy of Kölliker's ('76) Fig. 19. gd., Gelber Dotter. wd., weisser Dotter. bd., ungefurchter Bildungsdotter. s', grosses Segment. s, kleines Segment. k, Kugeln.

cells is completely cut off from the "Bildungsdotter." They form a layer 0.14 mm. in depth in the center. A section through a later stage in the development of the hen's egg is shown in Kölliker's Figure 22, (Fig. 42) where "die Dicke der durchfurchten Stelle in der Mitte des Keimes gerade noch einmal so dick war, als in dem früher beschriebenen Falle (Fig. 19), nämlich 0.28-0.30 mm." "Somit greift die Durchfurchung indem sie weiterschreitet in der Mitte der Keimschicht immer mehr in die Tiefe, wie schon Oellacher dies vermuthet hat, und erreicht am Ende nahezu die Grenze der Lage die in der Fig. 19 mit bd als ungefurchter Bildungsdotter bezeichnet ist." (Kölliker, '76, p. 74.)

Kölliker suggests that the adding of cells from below may be by a process similar to the adding of cells to the central part from the marginal segments, *i. e.*, the nucleus of a marginal segment divides and the central end of the segment containing one of the daughter nuclei is cut off and becomes a "Furchungskugel." The other daughter nucleus passes into the marginal segment, and so on until finally the part of the marginal segment left over changes into a "Furchungskugel." And so, according to Kölliker, the first appearing "Furchungskugeln" are never completely cut off from the unsegmented "Bildungsdotter" below, but nuclei, sisters to those in the first layer of cells, pass down into the "Bildungsdotter." Here nuclear division takes place, and cells are organized around the upper daughter nuclei, thus forming the second layer of cells in the center of the blastodisc, while the lower daughter nuclei are left deeper

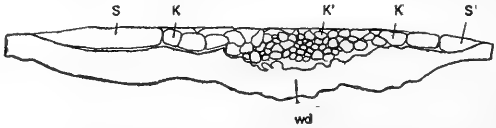


Fig. 42.—Copy of Kölliker's ('76) Fig. 22. Senkrechter Schnitt durch die Furchungsstelle eines Hühnereies aus dem Uterus. s, grosses Segment; s′, kleines Segment; k, grosse einschichtige Randkugeln; k′, kleinere Kugeln aus der Mitte geschichtet; wd, weisser Dotter.

in the "Bildungsdotter." And thus cleavage proceeds downward until finally the last remaining nucleated portions of the "Bildungsdotter" change over into "Furchungskugeln."

The Segmentation Cavity of the Pigeon's Egg is homologous with that of other vertebrate eggs.—But in the pigeon's egg, I do not find any such deepening of the center of the blastodisc. The change from one to several layers of cells is by a process *exactly* like that of the teleost egg. (See Agassiz and Whitman, '84, Fig. 2, and Wilson, H. W., '91, Figs. 16, 17, 18, and 19.) The blastodisc of the pigeon's egg becomes stratified by horizontal cleavage planes arising above the first horizontal cleavage, *i. e.*, above the level of the plane which limits the cell *b* below (Fig. 5). Nuclei are never found in the central part below the level of the horizontal cleavage under the cell *b*,—at least not until a much later stage.

In the description of the early development as given in the first part of this paper, it was pointed out that the position of the segmentation cavity may be indicated as early as the maturation stage, and certainly by the depth of the first vertical cleavage. And a comparison of sections of eggs of successive stages as represented by Figs. 2, 3, 4, 5, 6, and 11 (these figures are drawn to the same scale) shows the depth of the blastodisc to be constant in cleavage stages.

VI. The Periblast of the Bird's Egg Compared With the Vegetative Pole of Holoblastic Vertebrate Eggs.

The position of the segmentation cavity as I have found it in the pigeon's egg, presents an exact parallel of the bird's egg, in this respect, with other vertebrate eggs. And it supports the conception that the unsegmented part of the bird's egg (periblast plus yolk) is homologous with the cells at the vegetative pole or holoblastic vertebrate eggs. To the unsegmented part of the bird's egg His ('00) applied the physiological term "Lecithoblast." "In den dotterarmen Eiern der Säugethiere geht nach Ablauf der Furchung der gesammte Eiinhalt in der Bildung von Keimzellen auf. Anfangs zeigen die Blastomeren gewisse Unterschiede der Grösse und des Deutoplasmagehaltes, indessen kommt es nicht zur Bildung besonderer Dotteransammlungen. In Eiern mit reichlicherem Dotter sind die Vorgänge complicirter: Entweder greift die Furchung trotz des Dotterreichthums durch den gesammten Eiinhalt durch, oder es vollzieht sich eine räumliche Scheidung zwischen dem Keimplasma und dem unorganisirten Dotter.

"Die durchgreifende Furchung dotterreicher Eier kennen wir bei Amphibien, Ganoiden und Cyklostomen. Sie vollzieht sich in den verschiedenen Abschnitten der Eier ungleich rasch, in der oberen Hälfte rascher als in der unteren. Letztere, in der vom Anfang ab die Dotterplättchen reichlicher angehäuft sind, besteht noch aus grösseren Blastomeren, wenn die in der oberen Hälfte entstandenen kleinen Zellen sich bereits anschicken, Keimblätter zu bilden und in zunehmender Flächenausdehnung die untere Hälfte zu umwachsen. Die umwachsene Blastomeren-

masse betheiligt sich nicht an der Keimblattbildung, sie erhält sich als ein mehr oder minder compacter, mit dem Hypoblast in Verbindung bleibender Klumpen, der zunächst eine Dotterreserve bildet.—— Bei den genannten Eiformen ist übrigens von Anfang ab die Scheidung von Plasma und Dotter eine unvollkommene. Daher

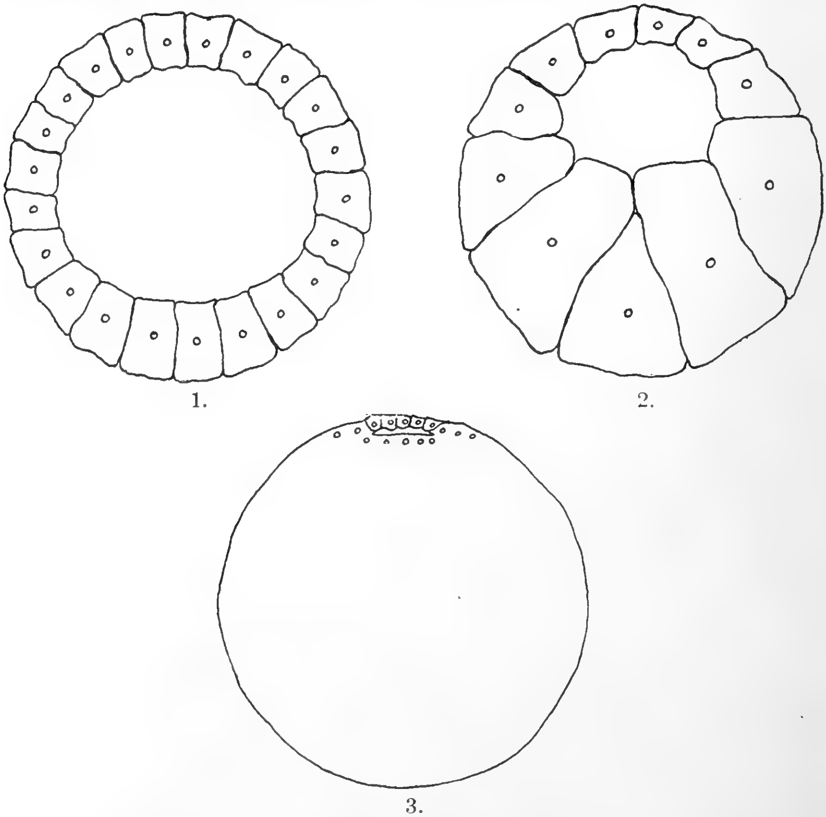


Fig. 43.—Diagrams of sections of three vertebrate eggs. 1. Amphioxus. 2. Frog. 3. Pigeon.

sind die Keimblatt- und die Organzellen noch während geraumer Zeit mit grösseren oder kleineren Dotterplättchen beladen.

"Zu einer räumlichen Scheidung von Keimplasma und unorganisirtem Dotter kommt es bei den sog. meroblastischen Eiern der Knochenfische, Selachier, Reptilien und Vögel. Am reinlichsten

vollzieht sie sich bei denen der Knochenfische, deren Blastomeren frühzeitig von körperlichen Dotterbestandtheilen frei erscheinen." (His, '00, p. 187.)

Three diagrams (Fig. 43) represent median vertical sections of the egg of amphioxus, the frog, and the pigeon. In amphioxus, the cells at the vegetative pole are but slightly larger than those at the animal pole, and the segmentation cavity is nearly central. In the amphibia, the segmentation cavity is nearer the animal pole, and the vegetative cells are large, but are separated from each other.

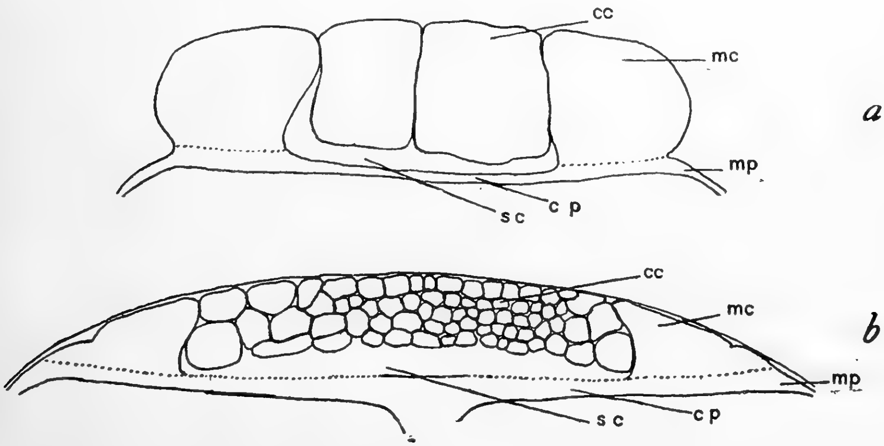


FIG. 44.—Diagrams of transverse sections of the eggs of (a) ctenolabrus, and (b) the pigeon. A is copied from Agassiz and Whitman ('84) Fig. 2. I have inserted the dotted lines to indicate the region from which cells are added from the periblast to the blastodisc. mc, marginal cell; cc, central cell; mp, marginal periblast; cp, central periblast; sc, segmentation cavity.

In the bird, the segmentation cavity is much further removed from the vegetative pole, and in relation to the size of the egg it is too small for comparison with the homologous cavity in the other eggs. The cells of the vegetative pole are not separated from each other, but they are the syncytial periblast. The cytoplasmic portion of the cells is confined to the animal pole, while their yolk contents occupy the entire remaining part of the egg. In Ctenolabrus cells are added to the central area from the marginal cells until the end of the period of cleavage (Agassiz and Whitman, '84, Fig. 5). The

dotted lines in the diagram (Fig. 44a) indicate the region of the periblast from which cells are added to the blastodisc. In the pigeon, however, this region is of greater extent (Fig. 44b) and cells are added to the blastodisc from the central periblast. Only the central part of the latter does not contain nuclei, and does not proliferate cells upward. I must explain Kölliker's description of the deepening of the blastodisc in this way. According to his description, it is impossible to homologise the cleavage of the bird's egg with that of other vertebrate eggs. Probably the deeper cells of the germinal area of Duval's ('84) Figs. 7 and 8 are derived from the periblast, and are to be regarded as holding the same relation to the cleavage of the blastoderm as those added from the zone of junction to the blastodisc in the teleost egg.

Summary.

A summary of the behavior of the supernumerary sperm nuclei during the first twelve hours after fertilization was given on page 18 and need not be repeated here.

1. *Polyspermy.*—Polyspermy has been reported in the Bryozoa, Holothuroidea, Insects, Selachians, Amphibians, Reptiles, Birds, and in one of the Gnetales. Polyspermy seems to take place because of the lack of protection against it. The migration of the supernumerary sperm nuclei in the pigeon (and perhaps in other forms) is a response to the attractive influence of the unfertilized cytoplasm in parts of the egg distant from the egg nucleus. The supernumerary sperm nuclei perform no important function in the egg; their presence is a matter of chance; and they are not essential to normal development. The accessory cleavage is comparable to the segmentation of fertilized enucleated egg fragments.

2. *Area of Primary Cleavage.*—The blastodisc and periblast are differentiated in the surface view in the maturation stage. The early cleavage planes bear no constant relation to the axis of the future embryo. Cleavage is not always asymmetrical. In the surface view there are three concentric areas: (1) the central, (2) the marginal area, and (3) the periblast. Cells are cut off centrally from the marginal cells and are added to the central area until the former

are used up, and then there are only the central area and the periblast. The marginal cells constitute a zone of junction between the blastodisc and the periblast.

3. *The Segmentation Cavity.*—The first horizontal cleavage plane marks the position of the segmentation cavity. The blastodisc becomes stratified by horizontal planes arising above this one, and the bird's egg in this respect is homologous with other vertebrate eggs.

4. The periblast of the bird's egg is the homologue of the cells of the vegetative pole of holoblastic vertebrate eggs.

LITERATURE.

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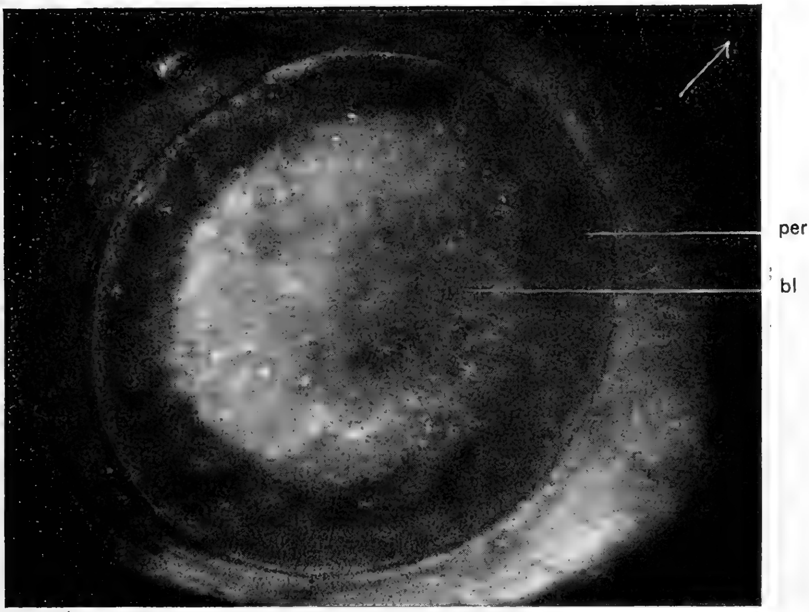

FIG. 45.—Photograph of a pigeon egg in the maturation stage. 11.30 p. m., 3½ hours after fertilization. The photograph was taken with reflected light from the whole mount. per, periblast. bl, blastodisc.

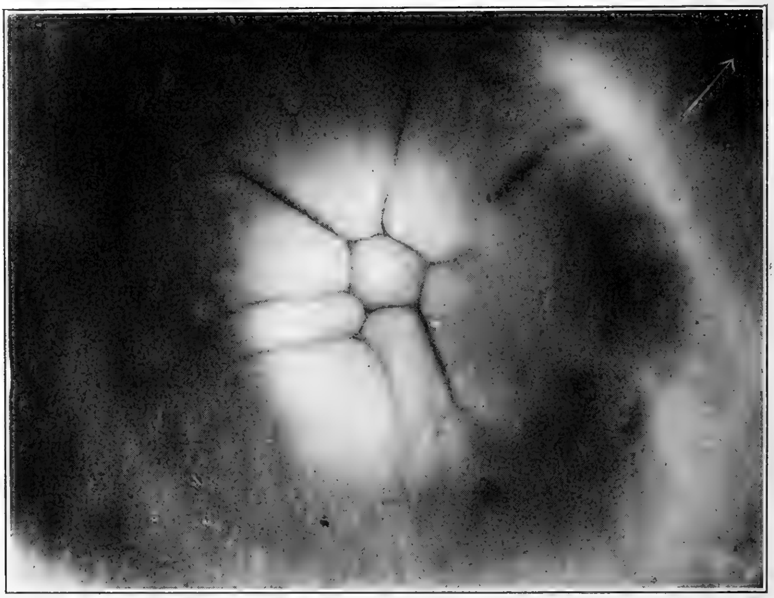

Fig. 46.—Photograph of a pigeon egg, eight and one-fourth hours after fertilization. 4.15 a. m. Killed and photographed in 5 per cent. formalin in normal salt solution.

Fig. 47.—Photograph of pigeon egg at 2.30 a. m., about six and one-half hours after fertilization. Killed and photographed in 5 per cent formalin in normal salt solution. Notice the vacuoles in the yolk surrounding the blastoderm.

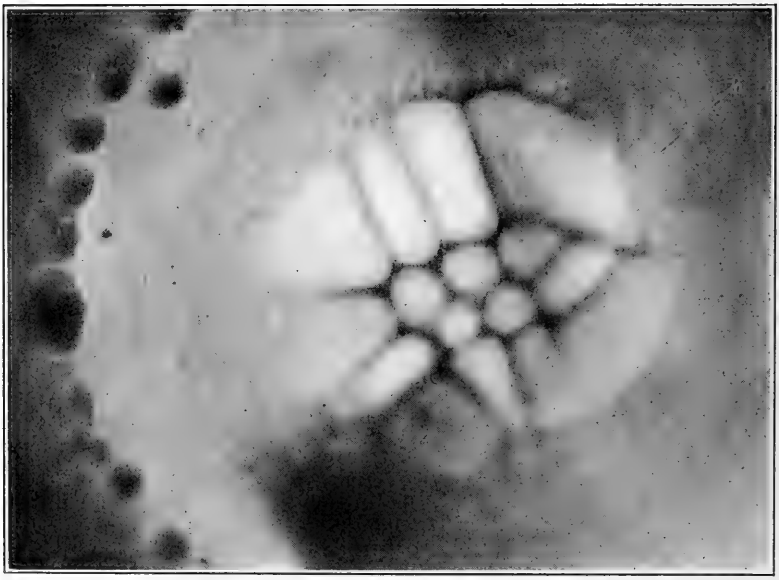

Fig. 48.—Photograph of a pigeon egg seven and three-fourths hours after fertilization, 3.45 a. m. The anterior side of the blastoderm is toward the point of the arrow.

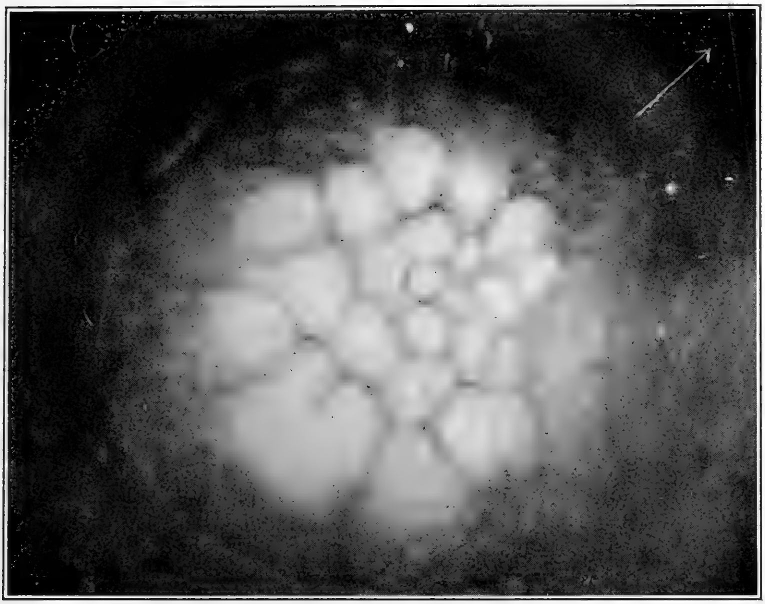

FIG. 49.—Photograph of pigeon egg. Bird killed at 5 a. m., nine hours after the estimated time of fertilization. Egg was in salt solution when photographed.

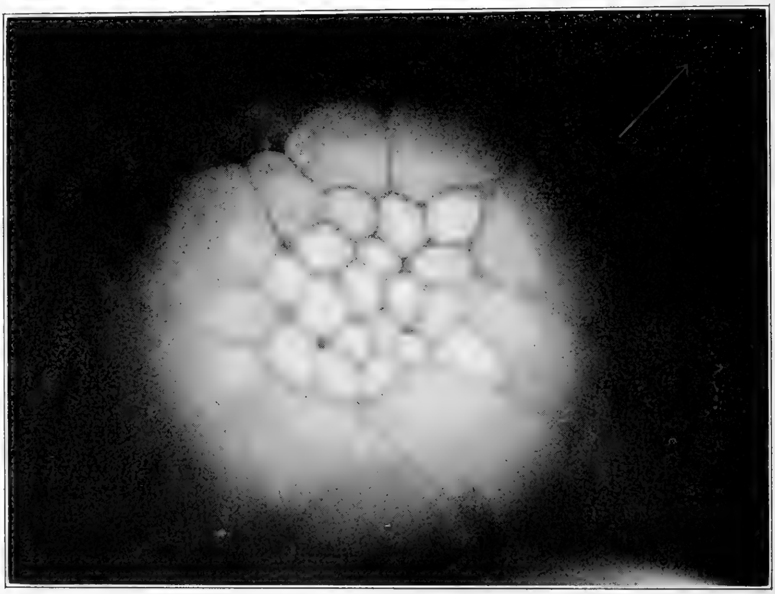

Fig. 50.—Photograph of a living pigeon egg. The bird was killed at 6 a. m., ten hours after fertilization of the egg. The photograph was taken through a window in the shell at 6.45 a. m. Then the egg was incubated until 11 a. m., when it was killed. A photograph from the whole mount of the later stage of the egg is shown in Fig. 47.

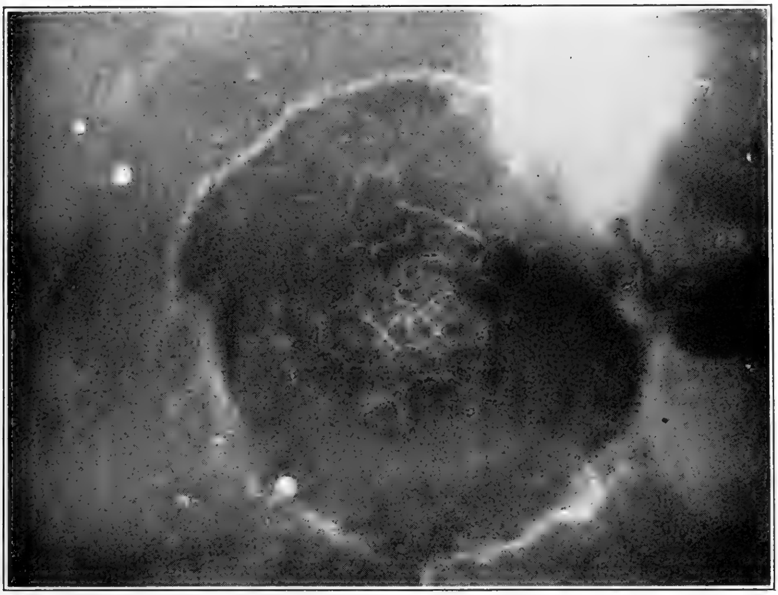

Fig. 51.—Photograph of a pigeon egg at 11.00 a. m., fifteen hours after fertilization. An earlier stage of the same egg is shown in Fig. 50. This photograph is taken from a whole mount, with reflected light. The light areas at the anterior and right side are due to breaks in the yolk. The light irregular circle is caused by a crack in the material.

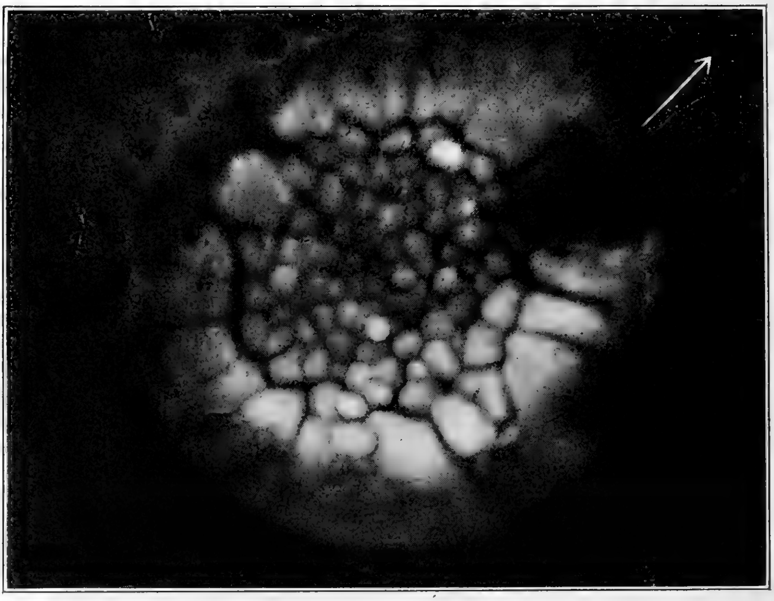

Fig. 52.—Photograph of pigeon egg, eleven hours after fertilization, 7.10 a. m. The point of the arrow indicates the anterior side.

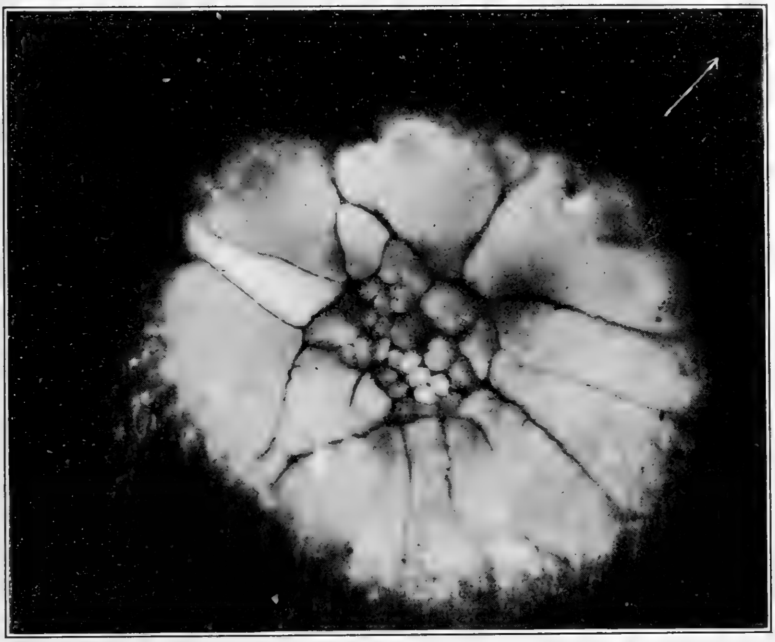

FIG. 53.—Photograph of a pigeon's egg at 7.00 a. m., about eleven hours after fertilization.

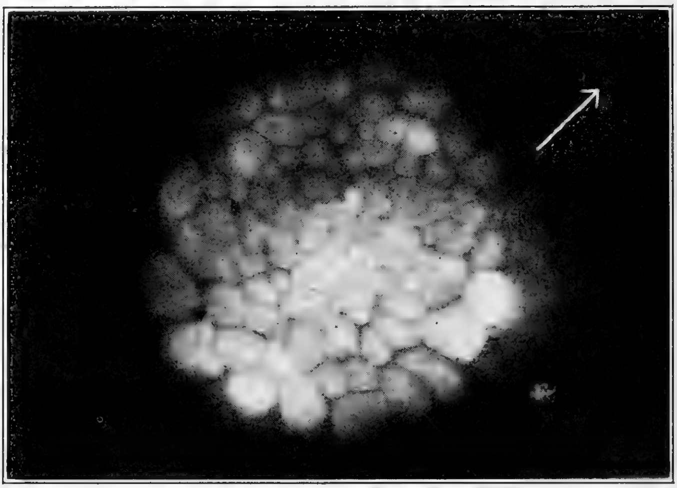

FIG. 54.—Photograph of pigeon's egg thirteen and one-half hours after fertilization, 9.30 a. m. Anterior side of blastoderm toward point of arrow.

GASTRULATION IN THE PIGEON'S EGG—A MORPHOLOGICAL AND EXPERIMENTAL STUDY.[1]

BY

J. THOS. PATTERSON.

TABLE OF CONTENTS.

I. Introduction.

In view of the fact that the bird has long been the classic type in the field of embryological research, it is surprising that the question of the origin of the entoderm[2] in this form should have

[1]From the Department of Zoölogy, University of Chicago.

[2]The terms entoderm, gut-entoderm, and invaginated-entoderm will be used synonymously throughout this paper.

remained unsatisfactorily answered. Nearly all recent writers acknowledge that the problem is far from being solved. Thus Nowack, writing in 1902, admits that he has failed to make clear the exact manner in which the entoderm takes its origin. He says, "Ich bin leider nicht in der Lage, auf Grund meiner Präparate eine absolut sichere Erklärung über die Entstehung des inneren Keimblattes zu geben. Das aber kann ich mit aller Bestimmtheit behaupten, dass das Entoderm nicht als eine Einstülpung am Rande des Blastoderms entsteht, wie es nach Duval der Fall sein soll."[3] The study of comparative embryology, nevertheless, would lead us to expect to find this germ layer arising by a process of gastrulation. Aside from a few descriptions of isolated stages, however, the theory of gastrulation is supported, by actual observations, only in the work of Duval ('84); but Duval's interpretation has been disputed on the ground that he was probably misled through the use of pathological material (Kionka, '94, Barfurth, '95, Schauinsland '99); and, as I have previously pointed out ('07, *b*), this author's work tends to support the idea of *delamination*. In this connection the statement of Hertwig ('03) is of special interest, in that he has often quoted Duval in support of gastrulation, but now says, "Der Darstellung Duval's war ich in meinem Lehrbuch längere Zeit gefolgt, halte sie aber jetzt nicht mehr für richtig und glaube, dass die in Fig. 482[4] am hinteren Rand der Keimhaut abgebildete Spalte zwischen Embryonalzellen und peripherem Dottersyncytium durch die Hartung oder beim Schneider künstlich erzeugt ist und mit einer Gastrulation nichts zu thun hat."[5]

Before a complete history of the early development of the bird can be written, therefore, it is necessary to give a detailed account, not only of gastrulation itself, but also of the stages preceding and immediately following it. Such an account is rendered possible by the fact that the writer has been able to secure a close series of stages covering this period of development.

The results recorded here are the outcome of a line of investiga-

[3] *Loc. cit.*, p. 27.
[4] Hertwig here refers to Fig. 8, Plate I, of Duval ('84).
[5] *Loc. cit.*, p. 861.

tion suggested to me by Professor Whitman, to whom I am indebted, not only for scholarly criticism, but also for his inspiring ideals of research. This paper is one of the series designed by Professor Whitman for the purpose of giving an account of the Natural History of Pigeons.[6] I also wish to express my gratitude to Professor F. R. Lillie for his assistance, and to the other members of the department for their interest in the work.

II. Material and Methods.

For the purpose of these studies the egg of the common pigeon offers several advantages over that of any other bird. (1) Its small size makes it especially easy to handle in preparing sections. (2) The fact that this bird breeds readily in confinement renders it possible to secure absolutely fresh material. (3) Undoubtedly the greatest advantage, however, is that of being able to secure all the early stages of development in definite sequence. This is made possible by the regularity of the laying habits of the pigeon, which ordinarily lays two eggs for each sitting. The first is laid late in the afternoon, usually between four and six p. m., and the second between one and two p. m. on the second day following. Harper ('04) has shown that fertilization in the latter egg occurs shortly before it enters the oviduct, about four hours after the first egg is laid, that is, at about eight p. m. The second egg is, therefore, forty-one hours in passing down the oviduct. Hence, by killing birds at various hours in the interval between the two eggs a close series of developmental stages can be secured. Such a series is indispensable to the discovery of demonstrative evidence of gastrulation and to a correct interpretation of the attendant phenomena.

In fixing the egg I have followed the method employed by Harper, in that the whole yolk is fixed and hardened before any attempt is made to cut out an oriented block of yolk containing the blastoderm. For fixing, various reagents have been employed, but the picro-acetic mixtures have proved superior to all others and during

[6]The series so far embraces the following: Guyer ('00), Harper ('04), Blount ('07), Patterson ('07 b), Riddle ('08).

the past year have been used almost exclusively. It was found advisable to vary the percentage of acetic acid with the age of the blastoderm.

For the most part, Delafield's hæmatoxylin has been used for staining, although iron hæmatoxylin and carmine have been employed. In connection with the cytological work I have used the anilin dyes to good advantage.

In stages prior to the appearance of the primitive streak it is necessary to determine the orientation of the blastoderm before using the fixing fluid. Fig. I, the scheme for orienting, shows that the axis of the embryo meets the chalazal axis at an angle of 45° instead of at right angles, as is the case in the chick. For experi-

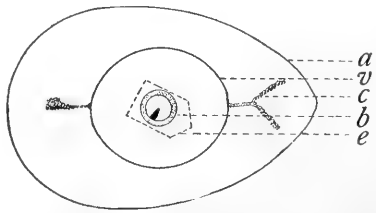

FIG. I. Scheme for orienting the blastoderm of the pigeon's egg in cutting sections. *a*, shell; *b*, blastoderm at the first appearance of the primitive streak; *c*, chalaza, which is sometimes double at the pointed end of the egg; *v*, vitelline membrane; *e*, wedge-shaped block of yolk containing the blastoderm which is cut out and embedded for sections.

mental work it is very important to know whether or not this angle is constant, particularly in experiments designed to demonstrate the movement of materials in the blastoderm. In order to determine this point, the record of about 200 eggs was kept, from which it was found that eight per cent show abnormal chalazæ. Of those with normal chalazæ, ninety per cent show the angle to be 45°, while in the remaining ten per cent it varies 1-5° from this angle. In the case of abnormalities the defect is usually found at the broad end of the egg, where the chalaza is either rudimentary or entirely wanting, or else its place of attachment to the vitelline membrane varies. In any of these cases the angle may vary greatly, even as much as 180°.

In most eggs the attachment of the chalaza to the membrane at the pointed end of the egg is much more intimate than at the opposite end. This, together with the fact that the position of the embryo upon the blastoderm is constant, has led the writer to believe that the chalazæ play an important rôle in maintaining the orientation of the egg in the oviduct. It seems very probable that the place for the attachment of the chalazæ to the vitelline membrane, at least at the small end of the egg, is determined in the ovary.[7]

From what has just been said it is obvious that eggs with abnormal chalazæ can be used neither for experimental work nor for sections, because the plane of section can not be determined. Consequently the utmost care has been taken in this work to detect and discard such eggs.

Special attention has been given to methods and means of experimentation, for it became increasingly apparent as the work progressed that there was need of a much more refined technique than that used by previous workers in this field. I have, in sterilization and in opening and closing the window in the shell, employed, in the main, the methods described in a previous paper (Patterson, '07, *a*), and they therefore need little explanation. A one tenth per cent solution of bichloride of mercury is used for sterilizing all instruments, except the operating needle, which is sterilized in alcohol. The window in the shell is made by the aid of a fine pair of forceps, and after the operation is performed, this opening is sealed with a piece of shell from a fresh egg, and a piece of sterilized cotton is placed over the closed window. The egg is then revolved until it is completely inverted, and thus, as the yolk turns, the blastoderm is brought uppermost into a normal environment. "Control" eggs show that by this method, not only is infection reduced to the minimum but also that the retardation in development which ordinarily accompanies this kind of work, is greatly reduced. After the operated egg has been developed for the desired time, it is taken from the incubator and the upper half of the shell removed. This allows one to determine the relation existing between the axis

[7]The writer has made observations and experiments to determine this point, but as yet they are incomplete.

of the embryo and that of the chalazæ, and consequently enables one to decide whether or not it is necessary to discard the egg.

For making the injury a No. 16 "bead" needle is employed. Although the diameter of this needle is small, yet it is entirely too large for very fine work, and so it was ground down to the desired diameter on an emery stone and then polished on a fine water-stone. By this means I have been able to secure a needle-point with a fineness of about 0.04 mm.

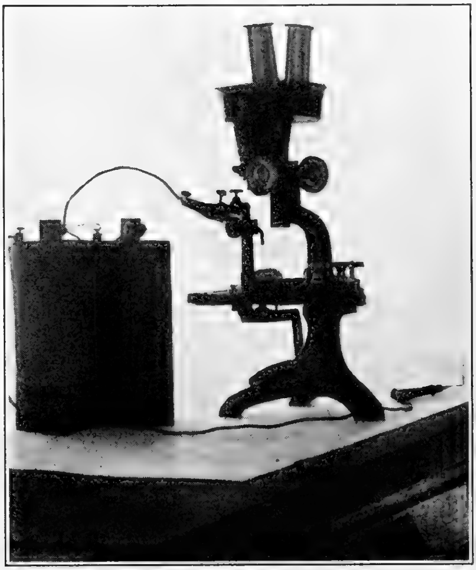

FIG. II. Apparatus used in operating.

By the aid of a special piece of apparatus, which is, in part, a modification of the one described by McClendon ('06), the needle is inserted in the blastoderm at the desired point. This apparatus is attached to a binocular and consists of an upright post fastened to the left end of the sliding bar of a Spencer mechanical-stage. Within the post the vertical end of an elbow is moved up and down by means of a rack and pinion. On the free end of the horizontal part of the elbow is a clamp which works on a universal joint. In operating, the needle-holder, which is connected with two dry battery

cells (see Fig. II), is held in the clamp, and by means of the universal joint the point of the needle is brought to bear directly over the blastoderm. To make the injury, the operator observes the magnified blastoderm (magnified 12.6 diameters) through the binocular, and with the right hand moves the needle horizontally by the mechanical-stage until the needle-point is directly above the place to be injured. The point is then inserted by adjusting the rack and pinion with the left hand, and the circuit is completed immediately by touching the second needle to the albumin. The extent of the injury can be regulated either by the number of battery cells included in the circuit, or by the length of time the current is allowed to run.

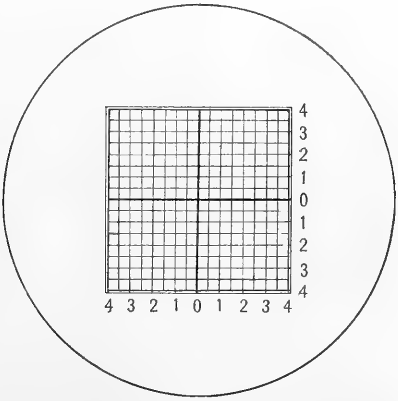

FIG. III. Eye-piece micrometer which is placed in one of the oculars of the binocular, and thus the blastoderm appears to the observer as plotted into small squares.

In order not to expose the blastoderm unduly while operating on early stages, it is highly desirable to have some quick and easy method for locating the place to be injured. This is done by using a net-micrometer, which is placed in one of the oculars, and thus the blastoderm is plotted into small squares. Two grades of micrometers are used, one ruled into 0.1 mm. and the other 0.5 mm. squares. A drawing of the latter kind is shown in Fig. III. In practice, the egg is placed in a depression at the top of a large cork, which, with the egg, can then be moved about on the stage of the binocular. In this way

the center of the blastoderm can be made to coincide with that of the micrometer. The numbers at the sides of the ruled area allow one to determine quickly the dimensions of the blastoderm, and at the same time the record of an injury in any quadrant is easily read in the terms of its co-ordinates.

In operating with this instrument there is a three-fold advantage

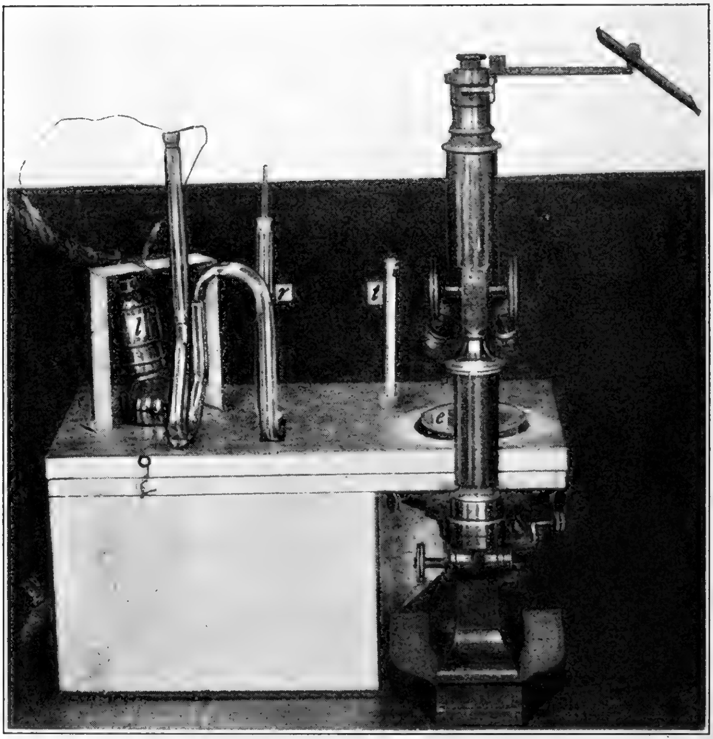

Fig. IV. Microscope-stage incubator used in studying the living egg.

over the free hand method: (1) the place to be injured is easily located; (2) the injury is made with mechanical precision; and (3) consequently the results obtained for any given set of operations are practically constant.

In connection with the experimental work as well as with the study of sections it is important to make direct observations on the

developing egg. In order to do this it was necessary to devise a *microscope-stage incubator,* a photograph of which is shown in Fig. IV. This apparatus is so constructed that it can be used with either a binocular or compound microscope, and, in case the latter is employed, camera drawings can be made of the object under observation.

The water in the incubator is heated by an incandescent lamp (l) controlled by an electric thermoregulator (r), which can be adjusted so that any constant temperature may be maintained in the region of the egg-cell (e).[8]

To study the developing egg, a hole is made in the shell and the blastoderm thus exposed is covered with fresh albumin. It is then placed in the egg-cell and nearly surrounded with sterile physiological salt solution, and the whole dish is covered with a thin glass plate. In this moist chamber eggs develop normally at least for several hours (in one case for thirty-three), and I have been able to study them, not only during the entire period of gastrulation, but also during many cleavage stages.

III. Gastrulation.

A. Study of the Developing Egg.

The individual variation in the development of pigeon eggs amounts ordinarily to about two hours, although in some cases it may reach as high as five hours. Owing to this variation it is difficult to set exact time limits to the process of gastrulation. In general, however, it may be said to occur between thirty-four and thirty-seven hours after fertilization. This conclusion is based on the fact that the youngest and oldest stages of gastrulation are usually found in eggs taken thirty-four and thirty-seven hours respectively after fertilization, and is further supported by the data gathered from a study of the developing egg. This does not mean that gastrulation in a given egg lasts for three hours. Indeed, in all probability not over two and a half hours elapse between the involution of the posterior margin and the closing of the blastopore.

[8]For a description of this incubator, see the Biol. Bull. for May, 1908, Vol. XIV, No. 6.

In order to understand fully the process of gastrulation, it will be necessary to consider somewhat in detail a series of stages covering a period of at least thirteen hours preceding the involution of the margin. Indeed, a knowledge of the entire history of cleavage is necessary; for all these early stages may be said to be preparatory to gastrulation. It does not fall within the scope of this paper, however, to consider these earlier periods. They have been studied and described by Miss Blount ('07). According to her account the supernumerary sperm nuclei disappear between ten and twelve hours after fertilization, and the marginal cells then "open peripherally and the periblast becomes organized with nuclei derived from the cleavage nucleus." From this time on the blastodisc increases in diameter by the addition of cells from the marginal and central periblast, cells which are "individualized" about the periblastic nuclei.

In the study of surface views of the developing egg, the changes observed between twenty and twenty-eight hours after fertilization are not very noticeable, for during the greater part of this period the blastoderm appears as a white opaque disc, there being no differentiation into areas opaca and pellucida. The disc, however, is not of equal opacity in all places, for the central region is more opaque than the marginal zone, these two parts gradually merging into each other. From the twentieth to the twenty-fifth hour[9] the margin of the disc is very irregular and gradually fades out into the surrounding zone of white yolk, which, for the most part, constitutes the "marginal periblast." From the twenty-fifth to the twenty-eighth hour the margin gradually becomes more regular and distinct, and at the same time the central opaque region increases rapidly, almost doubling its diameter. By the twenty-ninth hour the margin is still more regular and distinct, and the circumference of the disc is almost a circle (Fig. V, *A*).

Between the twenty-ninth and thirty-first hours the entire disc becomes more uniformily opaque, that is, the marginal region becomes thicker. This condition lasts but a few minutes, for almost immediately a small area lying just posterior to the center of the disc

[9]Throughout this paper the age of the egg will be designated by the number of hours that have elapsed after fertilization has taken place.

gradually becomes less opaque (Fig. V, *B*). This eccentrically lying region is the beginning of the area pellucida, and is brought about by the development of the subgerminal cavity, together with the thinning-out of that portion of the disc lying directly above this cavity. At first the boundary between the areas opaca and pellucida is very indistinct. In fact, this is more or less true throughout the entire period of gastrulation, and it is not until just a few hours before the egg is laid that a sharp differentiation between these two areas is established—a condition characteristic of the unincubated blastoderm.

Within forty-five minutes after its appearance, the area pellucida has practically doubled its diameter (Fig. V, *C*), this expansion taking place most rapidly toward the posterior margin. During the next two hours and a half the changes consist in an extension of the processes just described (Fig. V, *D-F*). In some cases the area pellucida extends almost to the posterior edge of the blastodisc, while in others it is difficult to determine from surface views the exact condition of this margin. Under high magnification, however, *the posterior edge of the disc is seen to differ from the rest of the margin, in that it does not blend into the surrounding yolk, but ends rather distinctly.*

At about thirty-four hours there occurs the most significant change yet observed. It is the appearance of an *indentation at the posterior edge of the blastodisc. This bay is the beginning of the gastrula-invagination, and often takes the form of a distinct marginal notch* (Fig. V, *G*). The edge of the disc included within the limits of the bay is to be regarded as the dorsal lip of the blastopore, and, owing to a slight opacity in this region, stands out in sharp contrast to the rest of the margin. During the next half hour the blastoporic margin changes from that of a notch to that of a broad shallow bay (Fig. V, *H*), finally becoming straight (Fig. V, *I*). This straight margin then becomes slightly rounded and less opaque (Fig. V, *J*), and at the same time the rest of the blastodermic edge is sharply defined. This change in the contour of the margin is due to the origin of the *region of overgrowth*, a structure that will be understood better from a study of sections.

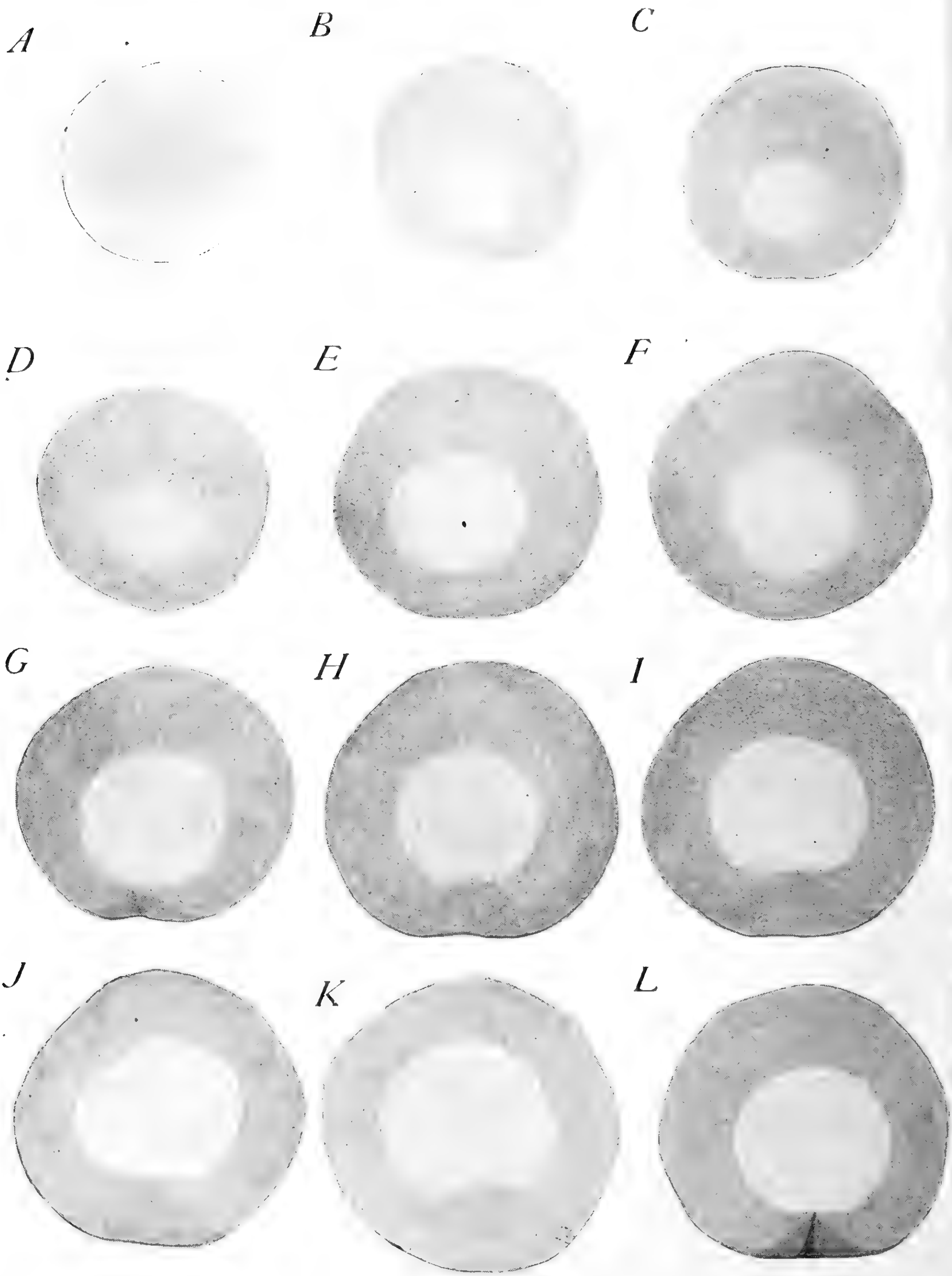

Fig. V.

Except in "rare" cases, all traces of the blastoporic bay are lost by the thirty-seventh hour, and the circumference of the blastoderm is again a circle. The only difference between the anterior and posterior halves of the blastoderm is found in the slightly opaque area lying between the areas opaca and pellucida at the middle of the latter half (Fig. V, *K*), and even this opacity usually disappears by the time the egg is laid, that is, by the forty-first hour. Hence the surface view of a freshly laid egg will give one no indication of the morphological difference existing between these two regions of the blastoderm.

Throughout the period of gastrulation the entire blastoderm grows less opaque—a change due to the progress made by the thinning-out of the blastodisc.

In Fig. V, *L* is shown one of two cases that have been observed in these studies, and that are of the greatest interest. Both of these blastoderms show a white opaque streak extending across the dorsal lip of the blastopore from the area pellucida to the posterior margin. This streak is narrow next the pellucid area, but posteriorly it becomes broader and its lateral edges are continuous with the right and left margins of the dorsal lip. The streak represents the line of fusion of the halves of the dorsal lip, for, as we shall see, these halves are moving from a lateral into a median position and

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Fig. V. This figure shows a series of drawings made by the aid of camera outlines from the developing egg. *A-D* are all from a single egg, and E-K from another. *L* is from a free hand sketch of a blastoderm taken about thirty-six hours after fertilization. The other drawings were made at the following periods: *A*, 29 hours; *B*, 31 hours; *C*, 31 hours 45 minutes; *D*, 32 hours 25 minutes; *E*, 33 hours 30 minutes; *F*, 34 hours; *G*, 34 hours 15 minutes; *H*, 34 hours 45 minutes; *I*, 35 hours 30 minutes; *J*, 36 hours 15 minutes; *K*, 37 hours 15 minutes. Owing to the individual differences in the development and size of various blastoderms at a given time, any one of the above surface views would not necessarily correspond to that of another egg taken at the time indicated. The two eggs from which these sketches were made were selected because they gave the appearance ordinarily met with at these times, as determined by the continuous study of several eggs throughout the period covered. It is not unusual to find a blastoderm, taken as much as five hours earlier than that figured in *B*, showing a pellucid area. All the sketches are × 12.

uniting along the middle line of the future embryo. This process of "concrescence" is operative in all cases, even though there is no perceptible streak in the majority of blastoderms. The question of concrescence will be considered in connection with the section on experimental studies.

B. Study of Sections.

a. Pregastrular Stages.

In the study of sections, it will suffice to begin by describing a blastodisc in what I shall term a late cleavage stage. A median longitudinal section (Fig. 1, Pl. I) shows that the blastodisc is thinner directly above the "Nucleus of Pander" than in other regions, except at the margin where it may be but one cell thick (Fig. 1, *a*). This thin marginal condition corresponds to the less opaque marginal zone seen in surface views from the twentieth to the twenty-eighth hours, and is brought about, as Miss Blount has shown, by the manner in which the blastodisc is increasing in diameter. External to the margin are periblastic nuclei about which cells are formed and added to the edge of the disc (anterior end of Fig. 1). This process may continue to such an extent that a row of several cells will be seen in section. This is not always the case, for periblastic nuclei are also present in the yolk lying directly beneath the thin margin; and about these nuclei, cells are organized and added upward to the disc, so that the margin may become more than a cell thick (posterior end of Fig. 1). Directly above the Nucleus of Pander, between the white yolk and the deeper cells of the disc, is the fissure-like segmentation cavity (*sc*), and between the edge of this cavity and the margin of the disc is a zone, in which the cells are open below to the white yolk. This region is more or less of a syncytium, in which cell boundaries are either wanting or very indistinct. It exists around the entire margin of the disc, and constitutes *the zone of junction.*[10]

[10]In my preliminary paper I used the term germ wall to designate this zone, but for the sake of unity it has seemed advisable to employ the term zone of junction instead. There is no objection to using this term to designate the entire zone at this stage, at least so long as one bears in mind the fact that the inner part of this zone is potential germ wall.

In connection with this stage (Fig. 1) it remains only to call attention once more to the thinness of the blastodisc above the segmentation cavity (*sc*). While there is some evidence in favor of the view that this thin condition existed from an early cleavage stage, yet, in the light of subsequent events, it lends itself to another interpretation, namely, that it is the beginning of a thinning-out process which will eventually succeed in producing a one-layered condition of the segmentation cells. In other words, all the cells of the segmented disc finally arrange themselves into an epithelial-like structure, the primary ectoderm. This interpretation for Fig. 1 receives support also from a study of several slightly younger stages, which show the disc to be from three to five cells thick in the central region.

As we have seen in surface views, the thinning-out does not begin exactly in the center of the disc (Fig. 1), but slightly posterior to this place, and then spreads in all directions but with more rapidity toward the posterior margin. This thinning-out evidently brings about a rapid centrifugal expansion of the disc, for there is no other period in the early history of the blastoderm in which there is such a rapid increase in the surface area, as occurs during the time when the thinning is at its maximum.

Coincident with the thinning-out, but not connected with it, another important process makes its appearance, that of the interruption of the posterior zone of junction. This interruption is associated with the degeneration of the periblastic nuclei beneath the zone of junction. The presentation of the facts upon which this conclusion is based must be deferred until more advanced stages have been described.

Let us consider next a series in which the progress of the thinning-out as well as that of the interruption of the zone of junction can clearly be seen. A median longitudinal section of such a stage is shown in Fig. 29, Pl. IV. The blastoderm from which this photograph was made is considerably in advance of that of Fig. 1 and would correspond to stage *D,* Fig. V. In addition to the progress made in the thinning-out and the interruption of the posterior zone of junction, the more important changes are (1) the great increase in the number of cells, and (2) the extension of the segmentation

cavity, which we may now call the subgerminal cavity.[11] At the posterior end (Fig. 29, *p.*) the blastoderm is only a single cell thick, but towards the anterior it gradually increases in depth. Although the anterior fourth of the disc is at least six cells deep, yet distinct layers can not be made out, but the cells are more or less loosely arranged. In the enlarged drawing of the anterior end the details

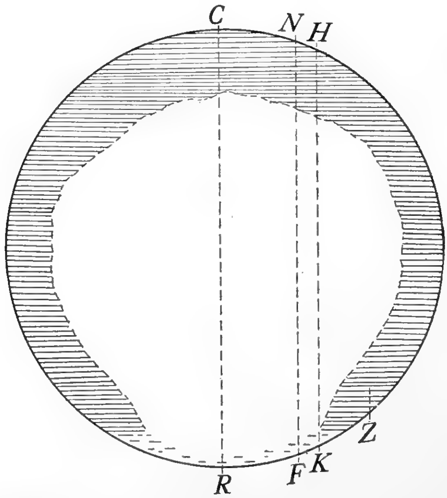

Fig. VI. A diagrammatic reconstruction from camera drawings of the sections of a blastoderm taken about thirty-three hours after fertilization. The lines *CR*, *NF* and *HK* are the planes of sections of Figs. 29 (or 2 and 3), 4 and 5 respectively. The zone of junction (*z*) is all but completely interrupted at the posterior margin. × 27.2.

of the zone of junction are shown (Fig. 2, Pl. I, *z*). Cells in every stage of formation are present, and at *ce* is one completely formed about a periblastic nucleus, and toward the center are several others undergoing the same process. The whole region from the letter *z* to the left end of the drawing is a syncytium—a region containing many periblastic nuclei.

[11]Although the term subgerminal cavity is here used in the sense in which it is usually employed, namely, to designate an enlarged segmentation cavity, yet it should be said that from the standpoint of comparative embryology, it has little or no significance.

At the posterior end of this same section (Fig. 3) an entirely different condition is found. Aside from the thinness of the margin, the almost entire disappearance of the zone of junction is the most characteristic feature. The only remnants of it are the degenerating periblastic nucleus (*pn*) and the single cell which is about to arise from the yolk (*ce*). For a considerable distance on either side of the posterior portion of this section the only normal periblastic nuclei visible are the few which are in the last stages of acquiring distinctly outlined cell limits. All other nuclei are in some phase of degeneration. About twenty-five sections to either side of the median line, however, the uninterrupted zone of junction is again found. In Fig. 4, which is taken twenty sections to the right of the center, two normal nuclei are forming cells about them (*ce*), and to the left of these there is a completed cell. Two degenerating nuclei are also present (*pn*). Five sections farther to the right, the first indication of a true zone of junction is found (Fig. 5). The zone here is very narrow, but still farther to the side it becomes much wider (see Fig. VI).

From this time on the thinning-out of the blastoderm and the interruption of the posterior zone of junction make rapid progress, until about thirty-one to thirty-three hours after fertilization, when the zone is completely interrupted for a distance of 70-80 degrees (Fig. VII) (cf Fig. V, *F*). Comparing Fig. 29 with that of a longitudinal section of such a blastoderm (Fig. 30), it is apparent that the condition of the latter has been brought about as the result of processes already described in connection with the former, and consequently, the section ends posteriorly in a thin free margin (Fig. 14), with the zone of junction entirely wanting. In passing forward, however, one finds a gradual increase in the thickness of the blastoderm (Fig. 30). The subgerminal cavity, which has increased both in depth and extent, is occupied by many segmentation cells, which, for the most part, lie in a row near the floor of the cavity. The position of the cells is purely an artifact—a condition produced during fixation; for a study of this section under high power reveals the fact that the upper contour of every nucleated cell or group of cells lying in the cavity exactly corresponds to the under

contour of the overlying cells. This is especially clear in the photograph at the point marked x as well as in other parts of the blastoderm. Hence, if it were possible to view this section in the living condition, the subgerminal cavity would be seen to contain few or no nucleated cells; for all these cells would then be crowded up

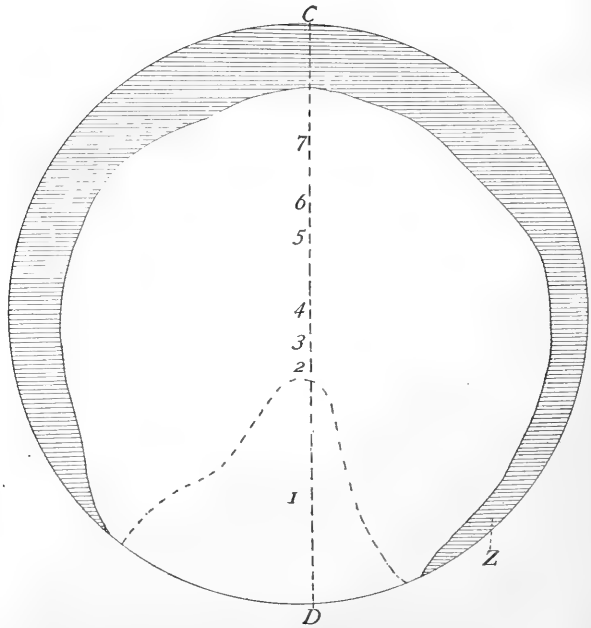

Fig. VII. A diagrammatic reconstruction of a blastoderm taken thirty-one hours after fertilization. It is farther advanced than the majority of eggs at this time. Numbers 1, 2, 3, etc., represent the regions of the blastoderm which are one, two, three, etc., cells deep, respectively. The broken line around "1" indicates the region where the depth is approximately one cell. The plane of the section for Fig. 30 is slightly to the left of line *CD*. $\times$ 27.2.

against the under surface of the blastoderm. Their present position within the cavity shows that they have loosened and sunk down during fixation. There would be, however, in the living condition, a few large non-nucleated yolk masses (m), as the present position of these bodies indicates that they have arisen out of the yolk lying beneath the floor of the cavity.

Since, in preparing sections, it is impossible to avoid entirely this artifact, it is important to recognize its significance.[12] A failure to do so might easily lead one to believe that after the completion of the primary ectoderm there would be many cells within the subgerminal cavity to form a "loose layer," and thus to attribute to this latter a possible origin of the gut-entoderm (delamination theory).

It is at about this stage of development (Fig. VII) that the initial step in gastrulation occurs, but before taking up that part of the description I must digress in order to make clear the probable significance of the method by which the avian blastoderm thins out. In this connection one naturally turns to the field of comparative embryology for suggestions, and here, if I mistake not, much evidence is found for an explanation of this interesting process. It will be necessary, however, to call attention to some well known facts in embryology, even at the risk of being somewhat tedious.

First of all, we may refer back to a holoblastic egg such as that of the primitive vertebrate Amphioxus. Here blastulation consists merely in an epithelial arrangement of the blastomeres to form a hollow sphere, and only the slightest difference in size exists between the blastomeres of the vegetative and those of the animal hemisphere—a difference, perhaps, anticipatory of a meroblastic condition.

In the egg of Petromyzon, which has greater meroblastic tendencies than the preceding but is still holoblastic, Hatta ('07) describes and figures a thinning-out of the upper hemisphere that begins approximately in the region where gastrulation is soon to appear and proceeds anteriorly, thus finally resulting in a one-layered condition of this hemisphere. The process, however, is not finished until just before the completion of gastrulation. He believes that this differentiation is brought about by the deeper cells pushing in between

[12]Many fixing fluids have been tried in an endeavor to overcome this artifact, but even in the best fixed series a few cells drop down, showing that they were not tightly wedged in between the upper cells. In one case I have succeeded in fixing an egg in an inverted position, and in this the subgerminal cavity is practically free from nucleated cells.

the more superficial ones. He says, "in the part where differentiation is going on, the cells of the outer row and those of the inner rows are found pushing between one another, and the layer of such condition passes over gradually into the part which has already become a true epithelium."[13] The expansion of the upper hemisphere necessarily brought about by this differentiation plays an important rôle in gastrulation.

Hatta points out the homology existing between the blastulation of Amphioxus and the differentiation of the micromeric layer into an epithelium in Petromyzon. He contends that since it is incorrect to speak of a "blastula" stage in Amphioxus before the blastomeres "are converted into the form of an epithelium," so in Petromyzon it is correct to speak of blastulation only when differentiation of blastomeres into an epithelium has begun. In regard to the latter form he writes, "In this case blastulation, as indicated by differentiation of the blastomeres into an epithelium, should be looked upon as being much delayed; it is still being carried on during the whole period of the gastrulation and is finished only a little earlier than the latter process. In other words, the two processes, blastulation and gastrulation, overlap each other to a great extent in the period of their occurrence. The prime cause of this belated mode of development is indisputably due to delay of segmentation on account of an enormous accumulation of yolk within the ovum."[14]

Without referring to the various eggs showing intermediate conditions, we may consider next the thinning-out in an egg in which the accumulation of yolk within the ovum is carried almost to the extreme, that is, in a meroblastic egg such as that of the Selachian *Torpedo* (Zieglers, '92), or *Pristiurus* (Rückert, '99). In *Torpedo* Ziegler figures and describes a "blastula" stage in which the posterior portion of the blastodisc is differentiated into a single-layered epithelium, while anteriorly it gradually increases in depth and the cells are not arranged in the form of an epithelium. At this stage, invagination of the thin posterior margin begins and soon after, the differentiation (thinning-out) extending both anteriorly

[13] *Loc. cit.*, p. 24.
[14] *Loc. cit.*, p. 35.

and laterally reduces the entire central region of the blastodisc to a single layered epithelium. Concerning this extension of the differentiation, he writes as follows: "Die epitheliale Schichte ist jetzt in ziemlich gleichmässiger Weise an der ganzen Oberfläche des Blastoderms zur Ausbildung gekommen (Fig. 2). Offenbar sind also die Zellen, welche in dem früheren Stadium (Fig. 1) den dickeren Theil des Blastoderms bildeten, in dieses epitheliale Blatt eingetreten, indem die tieferen Zellen sich aufwärts nach der Peripherie bewegten und sich dem Epithel einordneten; daher nahm die epitheliale Schicht beträchtlich an Ausdehnung zu und in Folge dessen hat das Blastoderm jetzt eine grössere Länge und Breite und ist ein Umstülpungsvorgang am Hinterrande des Blastoderms eingeleitet worden."[15]

Almost the same words could be employed in describing the changes which occur in a pigeon's blastoderm after it has reached a stage corresponding to that shown in Fig. VI. Hence, the process of thinning-out of the avian blastoderm, as well as that of the selachian, is to be regarded as homologous with the process of blastulation in Amphioxus and Petromyzon. There will be, undoubtedly, a wide difference of opinion as to the advisability of using the term "blastulation" to describe this process; for the term blastula has been employed for stages which cover a wide period of development. Ordinarily, however, it is used to designate that stage of development just preceding the gastrula-invagination—a stage in which the segmentation cavity is more or less enlarged. The result is that the so-called blastulæ of the various vertebrates have not the same morphological value.

As to when one should call the pigeon's egg a blastula, will depend on the criteria adopted. Using the term as it is variously applied among the different vertebrates, one might speak of a blastula from the eight-cell stage to the beginning of invagination, and, adopting Hatta's suggestion, even to the end of gastrulation. It is obvious, therefore, that the term could be used only in the most general way. I prefer to avoid it altogether, and for that reason, shall

[15] *Loc. cit.*, p. 58.

simply speak of the thinning-out process, by which I mean the differentiation of the cleavage cells into a single layered epithelium above the enlarged segmentation cavity (subgerminal cavity).

In this connection I must speak of the probable reason why the thinning-out process affects the posterior region of the blastoderm first. I have come to regard this as signifying that the posterior region is farther advanced in its differentiation than other parts. This interpretation is in harmony with the general law for the early development of the embryo, namely, that differentiation progresses from the head end backward. As we shall see later, it is in the posterior central part of the blastoderm that the head of the embryo will arise.

b. Gastrulation Stages.

(1) Invagination.

If the thinning-out were completed before the invagination began, the interpretation of the steps of gastrulation would be greatly facilitated. But such is not the case, for immediately following a stage such as shown in Fig. 30, the initial step in gastrulation occurs. This consists in the rolling under of the free posterior margin of the blastoderm. The reconstruction of a blastoderm in which the involution has just taken place is shown in Fig. VIII, and a surface view of a corresponding stage is seen in Fig. V, *G*. In this egg (Fig. VIII) the zone of junction is not essentially different from that seen in Fig. VII, except at the anterior inner margin, where a portion of it has given rise to a partial germ-wall (*gw*). The numbers scattered over the figure indicate the relative depths of the various regions. Thus in the central area, the blastoderm is thinned-out to one or two cells, while the marginal parts are much thicker, varying from two to four cells. In the extreme posterior is shown the region covered by the invaginated entoderm (*E*).

The posterior portion of an oblique section passing through the region of invagination is represented in Figs. 32 and 15. At the extreme posterior is a cavity (Fig. 15, *c*) which is bounded above by the vitelline membrane and below by the yolk, or ventral lip of the blastopore. In reality the cavity is but a portion of the blasto-

pore (b), which passes beneath the dorsal lip (d) to become the archenteron (ac). Directly above the archenteron is the invaginated entoderm (e), and just in front of the anterior limit of this is a portion of the subgerminal cavity (sg), above which the blastoderm is two cells thick, but anterior to which it is three thick. Owing to the obliquity of the plane of section, the wrong impression is given as to the condition of the blastoderm directly in front of the central

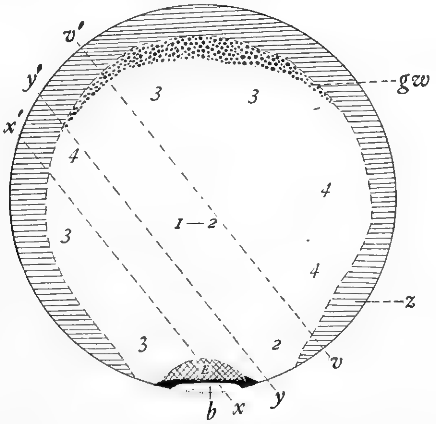

FIG. VIII. A diagrammatic reconstruction of a blastoderm taken thirty-four hours after fertilization, or seven hours before laying. Invagination has just taken place and the entoderm (E), a tongue-like process, is starting to grow forward through the subgerminal cavity. As indicated by the numbers, the blastoderm is thinned out to one or two cells deep in the central part, while around the anterior and lateral margins it varies from two to four deep. The anterior inner edge of the zone of junction is differentiated into a germ wall. In this, as in the other reconstructions, the ectoderm is not represented.

part of the invaginated region. If the series had been cut parallel to the longitudinal axis of the future embryo, a median section would have been diagrammatic in clearness, that is, it would show what we should expect to find in case of a true involution. The condition of the central part of the blastoderm, however, can be inferred from the photograph shown in Fig. 49. On either side of the invaginated region the posterior ends of the sections terminate with thin free margins (Fig. 16), and differ from those in the invaginated area, therefore, in having no cavity posteriorly.

I have said above that invagination takes place by a turning or rolling under of the free margin. It is important to show that there is a plain rolling under, and the following facts are offered as proof. First, as regards the morphological evidence; I think it is clear from the above description that this line of proof strongly supports the conclusion. There is no other explanation for the appearance of a cavity just beyond the posterior margin (Fig. 15, *c*) than that it was brought about as the result of the rolling under of the edge and of the simultaneous forward growth of the involuted cells.

This conclusion can be tested experimentally; for an injury made on the edge of the thin posterior margin (Fig. IX) just previous

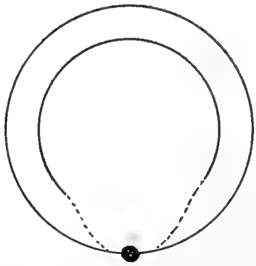

Fig. IX. Scheme for the operation in Experiment I.

to gastrulation ought to be carried down beneath the blastoderm during the course of further development, that is, it ought to be found in the entoderm.

Experiment I.

The operation was made thirty-three and one-fourth hours after fertilization, and the egg was then incubated for three and three-fourths hours. The result of the injury is shown in the posterior end of the median section (Fig. 66). There is a distinct dorsal lip, in which the deeper portion shows the cells affected by the operation. In the vitelline membrane, a short distance posterior to the dorsal lip, is the break made by the operating needle (at *op*). All of the injured cells are found in the entoderm, while the ectoderm is well differentiated almost back to the end of the section. Just ante-

rior to the dorsal lip the entoderm is almost wanting (Fig. 67). In an uninjured blastoderm at a corresponding stage of development the entoderm in this region is very thick (see Fig. 37). It is clear

TABLE I.

	Ser.	Age.	Antero-post. diameter.	Trans. diameter.
Late pre-gastrular stages....	304	31 hrs.	2.857 mm.	2.857 mm.
	427	31 "	3.333 "	3.333 "
	346	32 "	3.428 "	3.428 "
	411	33 "	3.411 "	3.411 "
	326	34 "	3.809 "	3.809 "
Gastrulation stages.........	394	34 "	3.333 "	3.619 "
	342	34 "	3.285 "	4.238 "
	368	34 "	2.571 "	2.667 "
	370	34 "	3.428 "	3.524 "
	372	34 "	2.761 "	3.142 "
	190	35 "	2.860 "	3.333 "
	409	35 "	2.400 "	2.857 "
	164	36 "	2.860 "	3.333 "
	178	36 "	2.857 "	3.333 "
	188	36 "	2.860 "	3.048 "
	254	36 "	2.857 "	3.048 "
	330	36 "	2.952 "	3.333 "
	362	36 "	3.000 "	3.500 "
	406	36 "	2.400 "	2.857 "
	176	37 "	2.660 "	2.857 "
Early post-gastrular stages..	162	37 "	3.247 "	3.333 "
	256	37 "	3.333 "	3.429 "
	390	37 "	3.333 "	3.429 "
	332	38 "	3.428 "	3.428 "
	316	39 "	2.762 "	2.762 "
	166	40 "	3.524 "	3.524 "
	382	40 "	3.524 "	3.524 "

therefore, that while such an operation destroys most of the cells that are to give rise to the entoderm, yet the posterior margin is still capable of forming a rounded dorsal lip.

Measurements taken on the living eggs also can be interpreted in support of this view; for such data show that previous to and following gastrulation the blastoderm is approximately circular, while during gastrulation the antero-posterior diameter is always shorter than that of the transverse (Table I). This is what we should expect in case the margin actually involuted.

Owing to individual variation in the size of different blastoderms at the same stage of development, it is impossible to determine, from the above table, whether the antero-posterior diameter is actually shorter after the beginning of gastrulation than just preceding the

TABLE II.

	Egg 448.			Egg 440.		
	Time.	Ant. Post.	Trans.	Time.	Ant. Post.	Trans.
Pre-gastrular stages	6.00	2.857 mm.	2.857 mm.	6:00	3.510 mm.	3.510 mm.
Gastrulation stages	6.30	2.762 "	2.953 "	6:15	3.333 "	3.570 "
	7.00	2.857 "	3.047 "	6:45	3.451 "	3.689 "
	7.30	2.953 "	3.095 "	7:30	3.510 "	3.748 "
	8.30	3.142 "	3.238 "	8:15	3.570 "	3.748 "
Post-gastrular stages	9.30	3.242 "	3.242 "	9:15	3.808 "	3.808 "

same, or whether it is only relatively shorter in comparison with the transverse diameter. If it can be shown that the former alternative is the true one, then the evidence for a "plain rolling under" of the margin will be well nigh conclusive. This I have been able to do by studying the living egg and taking measurements of the same blastoderm at different periods of its development. In the above table are given the data from such measurements taken on two eggs.

Finally, the above interpretation for the origin of the entoderm is in harmony with the views of a large majority of the investigators who have worked on other groups of vertebrates. It is with the fish, however, that the most interesting and instructive comparisons are to be drawn. The large size of the selachian ovum,

together with the fact that this form is a more generalized type, would seem to indicate that the development of the avian egg ought more nearly to approach that of the selachian than that of the teleostean ovum, and so far as the thinning-out is concerned, it does; but as regards the involution of the margin it more closely resembles the teleostean type. Thus, Agassiz and Whitman ('84) state that in Ctenolabrus there is a "plain rolling under, or involution, as an initiatory step in the formation of the ring." However, they regard it more correct to describe the process "as an ingrowth, due both to a rapid multiplication of cells, and also to the centrifugal expansion of the ectoderm."[16] The ingrowing under layer in the pigeon's blastoderm with its free inner edge is in many respects comparable to the "ring" in the teleostean blastoderm, and is, therefore, to be regarded as a highly modified germ-ring. It is, of course, only a partial ring, in that but a small part (at most an arc of 70-80 degrees) of the margin invaginates, while in the ordinary teleost an invagination occurs around the entire margin. In the egg of the Toad-fish (Batrachus tau), however, we have an interesting modification of the germ-ring, a condition which can be understood best by quoting a part of the summary of Miss Wallace ('99), who has described the development of this ring. She writes as follows: "In the egg of Batrachus there is a centripetal growth of cells at the embryonic pole, the ingrowth having a voluted outline in sections. Around the remainder of the blastoderm there is not even the appearance of an invagination, but only a slight thickening due to an ingrowth of cells from the ectoderm, and a few loose cells which may represent a true germ-ring found as a layer in ordinary forms. The peripheral thickening gradually fades out, first at the anterior pole, until the last remnant is found in a few cells lying beneath the ectoderm, forming a linear streak from the posterior end of the embryo to the lip of the closing blastopore."[17]

We have in the egg of the Toad-fish a condition intermediate between such a form as Ctenolabrus and the Pigeon. The eggs of these three forms represent a series in which the differences in

[16] *Loc. cit.*, p. 68.
[17] *Loc. cit.*, p. 12.

development are measured by the relative quantities of yolk accumulated within the ovum. Thus in the Ctenolabrus egg, which contains the least amount of yolk, invagination occurs around the entire margin of the blastoderm, but stronger at the embryonic pole; in the Batrachus egg, which contains much more yolk than the preceding, there occurs only a slight thickening about the greater part of the margin as "the initiatory step" in invagination, this thickening soon disappears, and at the embryonic pole alone is there a true germ-ring formed; and finally, in the Pigeon egg, which is loaded to the extreme with yolk, invagination occurs at the "embryonic pole" only, the greater part of the margin lacking even "the initiatory step."

(2) *Middle and Late Gastrulation Stages.*

The entoderm after reaching a stage such as shown in Fig. 32 continues to grow forward through the subgerminal cavity as a tongue-like process. At the same time the thinning-out progresses anteriorly and laterally, ordinarily with sufficient rapidity to keep ahead of the advancing entoderm. This results in most blastoderms in the formation of a space just in front of the anterior limit of the entoderm. This space is but a part of the subgerminal cavity that is free from segmentation cells, the latter having passed upward into the differentiating ectoderm. In some few cases, however, no space is found and in such it is impossible to determine the anterior limit of the entoderm.

The posterior end of a median longitudinal section, in which the length of the invaginated layer equals about one third of the diameter of the blastoderm, is shown in Fig. 34. Only a part (about one-third) of the above mentioned space is included in the photograph. The ectoderm above the space, as well as posterior to it, is not yet differentiated into a single layer, but here and there the lower segmentation cells are seen apparently crowding in between the upper ones. A group of such cells is shown at *s*. The dorsal lip of the blastopore is much thicker than in Fig. 32, and the method by which it increases will be discussed in another connection. It

is sufficient to state here that in all probability it is not brought about alone by the multiplication of cells *in situ*.

A section lateral to the median line shows essentially the same conditions as Fig. 34, except that the entoderm does not extend so far anteriorly (Fig. 33, *e*).

The question must naturally arise in the reader's mind as to whether or not the upper layer is still rolling under at the posterior margin to give rise to the lower layer. The appearance of sections would seem to indicate that it is (*e. g.*, Fig. 33). The question can be tested, however, by experimentation, for if a rolling under is occurring, cells disturbed by an injury made on the extreme posterior margin of the dorsal lip, ought to be found later in the entoderm.

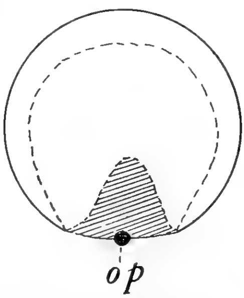

Fig. X. Scheme for operating in Experiment II.

Experiment II.—The scheme for such an operation is shown in Fig. X, and the result in Fig. 50. The injured cells are found immediately associated with the entoderm. This is especially clear in a transverse section through the affected region (Fig. 51). There is no evidence of an injury either in the ectoderm or mesoderm, and hence we must conclude that the affected cells have been brought to their present position by an actual rolling under of the posterior margin. Although this operation has been repeated several times with the above result, yet the position of the injury in the entoderm may vary in an antero-posterior direction; but this variation is easily accounted for by the fact that one can tell in the living egg only approximately the extent to which invagination has progressed.

If an injury be made in the same manner as above on slightly

older blastoderms the affected region is not found in the entoderm, but in the ectoderm and mesoderm, showing that the involution has ceased, and the further extension of the entoderm is now brought about by an ingrowth, in which cell division and the centrifugal expansion of the ectoderm play an important rôle. The latter two processes are doubtless factors in the extension of the entoderm throughout the entire period of involution, but they are not so conspicuous during the earlier stages of invagination.

We must now pass to a series in which gastrulation may be said to have reached its height, and one in which several structures and processes hitherto unnoted must be considered. The reconstruction of this series appears in Fig. XI. The tongue-like process of entoderm (E), the dorsal lip of the blastopore (D), the germ-wall (GW), and the zone of junction (z) were considered in connection with Fig. VIII, but they have all undergone important changes. Thus, the germ-wall extends almost around the whole inner margin of the zone of junction, and on the lateral margins its cells extend into the subgerminal cavity, within the edge of the area pellucida. This extension of cells is not due to an ingrowth from the inner edge of the germ-wall, but rather to the spreading of the subgerminal cavity by the liquefaction and fragmentation of the underlying yolk.

The changes in the dorsal lip consist in the growth of the right and left halves toward each other and their simultaneous fusion in the middle line, that is, in the plane of the longitudinal axis of the future embryo. A blastoderm in which the line of fusion was seen in surface view is shown in Fig. V, *L*. The movement of material is participated in by the more lateral parts of the margin, namely, the horns of the zone of junction, and as they move toward the median line they are at the same time being carried centrifugally by the expansion of the blastoderm, and in this way the fused halves of the lip are gradually being enclosed within the inner edge of the zone. The question of this movement of material will be fully discussed in connection with the description of experiments performed to throw light on the method by which the embryo arises.

The region of overgrowth (O), which is represented in the figure

as a crescent-shaped area extending around the anterior and lateral margins, is a structure hitherto not noted. It arises, however, at an earlier period than this, and consists in the outgrowth of the marginal cells beyond the zone of junction.

Besides the entoderm and the germ-wall cells (at the sides) there are many large yolk masses within the subgerminal cavity, and also a few of the lower segmentation cells that have sunk down from the

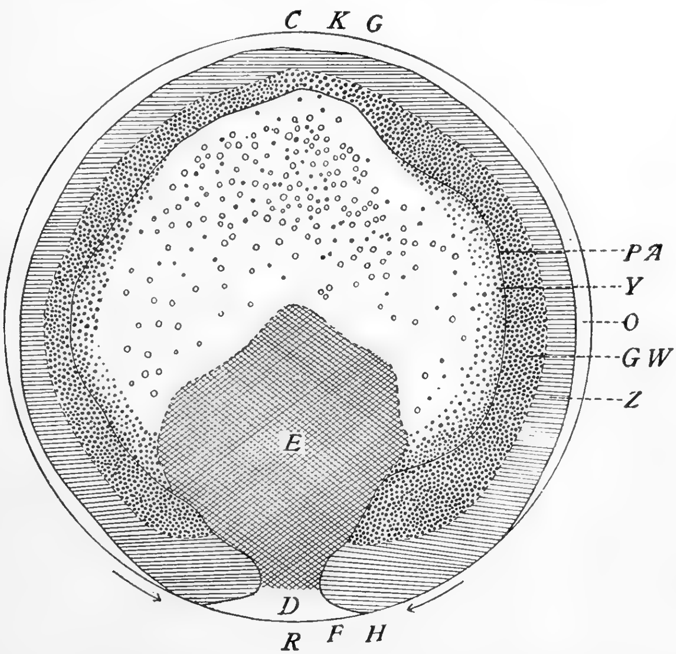

Fig. XI. A diagrammatic reconstruction of a blastoderm taken thirty-six hours after fertilization, or five hours before laying. It represents the ectoderm as transparent. *O*, region of overgrowth; *Z*, zone of junction; *Y*, germ-wall cells beneath which the subgerminal cavity has spread; *D*, dorsal lip of the blastopore; *PA*, outer boundary of the area pellucida; *E*, region covered by the invaginated or gut-entoderm. Lines drawn through *CR*, *KF*, and *GH* represent the planes of the sections illustrated in Figs. 35, 40, and 41 respectively. The anterior margin of the entoderm as here represented is only the average for the different lengths of entoderm as measured in the sections, from which the reconstruction was made. The arrows at the posterior margin indicate the direction of movement of the halves of the dorsal lip. × 27.2.

under surface of the differentiating ectoderm. That part of the subgerminal cavity lying beneath the entoderm (E) is to be regarded as the archenteron, which communicates with the blastopore by a narrow passage situated just below the dorsal lip (D). At its union with the deeper cells of the lip, the entoderm is very thick, but gradually thins out anteriorly, ending with a thin irregular margin slightly beyond the center of the blastoderm.

The changes described above can be made clearer by a study of longitudinal sections. Thus in the photograph of a median section (Fig. 35) the various regions are easily recognized, and in the enlarged drawing of the posterior end (Fig. 19) the dorsal lip is seen to be composed of compact cells, all of which are completely delimited by cell-walls. Directly above the lip there is no distinct ectoderm, but anterior to the point u it is well differentiated and in only a few places (s) are lower segmentation cells crowding upward into it. The entoderm at its union with the deeper cells of the lip is five cells thick, but anteriorly gradually decreases, finally ending with a free margin (Fig. 35, *e*—to the left). At this stage the entoderm can not be said to be a distinct layer, for its cells are arranged more or less into groups. In the archenteric cavity (ac), which lies between the entoderm and the yolk, are several large yolk masses, some of which are in the act of rising from the floor. Posteriorly the archenteron communicates by a narrow passage with the blastopore.

The conditions presented in this section very much resemble those of a corresponding section from a teleostean blastoderm in which invagination is well advanced. Thus Miss Wallace's ('99) Fig.5, Pl. III, not only compares very favorably with Fig. 19 in the appearance of the dorsal lip, but also as regards the method by which the entoderm grows forward; for I consider it better to describe the entoderm as now advancing anteriorly by a multiplication of its cells and their gradual arrangement into a single layer. This view is in accord with the account for Ctenolabrus as given by Agassiz and Whitman ('84). At this stage the main difference between the teleostean and pigeon blastoderms is that in the former the ectoderm is from three to five cells thick at the embryonic pole, while in the latter it is but one cell thick (Fig. 37). This difference

is doubtless to be accounted for by the fact that the teleostean embryo is precociously formed, that is, as compared with the formation of the avian embryo.

The conditions at the anterior end of this section (Fig. 18) are entirely different from those at the opposite end. First of all, the differentiation of the ectoderm into a single layered epithelium is not complete, for in many places the lower segmentation cells are crowding upward against its under surface, although some of them have sunk down into the cavity (*s*), but aside from these few the subgerminal cavity is entirely free from nucleated cells anterior to the fore end of the entoderm (*e*). There are found in the cavity only large yolk masses, some of which are disintegrating (*dm*).

The germ-wall is not well differentiated in this section, but in the sections to either side it is clearly defined.

The region of overgrowth (*o*) is a wedge-shaped process extending out from the zone of junction. The earliest stage in which this region has been observed is illustrated in Fig. 27, and is characterized by having no periblastic nuclei either beneath it or external to it, and by having a fine granular area just beneath its under surface.[18] This region arises when the thinning-out is at its maximum and at first is three or four cells thick, but later becomes reduced to a single layer of cells (Fig. 28).

The phyletic significance of this region is not clear. On the one hand, it might be compared to the overhanging margin of the selachian blastoderm, and thus be regarded as showing a tendency toward a "peripheral gastrulation." Its appearance in an unincubated chick blastoderm would favor this view (Fig. 65). On the other hand, the fact that it first arises at the anterior margin and is not a continuation of a dorsal lip (Fig. XI), would indicate that it was not comparable to the margin of the selachian blastoderm. The answer to this question, however, turns upon the view one takes as to the extent of the blastopore. I cannot agree with those in-

[18]In the series shown in Fig. 10 but a single nucleus was found beneath the overgrowth (Fig. 25), and this one had doubtless arisen from the nucleus lying below the zone of junction when that region formerly occupied the margin of the blastoderm.

vestigators (Haeckel, Balfour, Goette, and others), who have maintained that the entire margin of the avian blastoderm is to be regarded as the blastopore, for the evidence furnished by my material is conclusively in favor of the view that but a small part of the margin is the blastoporic region. The rest of the margin (overgrowth region) I regard therefore as a specialized region, rather than as a place where the upper germ-layer bends under to become continuous with the lower layer.

The three regions, overgrowth, zone of junction, and germ-wall, are all concerned in the spreading of the blastoderm over the yolk. Since the region of overgrowth has no periblastic nuclei either beneath or external to it, its spreading over the yolk can not be due to the addition of cells from the periblast, unless it be indirectly from the zone of junction. However, the fact that its cells are undergoing rapid division makes it almost certain that the spreading of the overgrowth is due to the multiplication of its own cells. This conclusion is strengthened by the fact that the cells are digesting the underlying yolk as indicated by the fine granular area.

As the overgrowth travels peripherally over the yolk, it is followed by the zone of junction, which in turn is differentiating, from its inner edge, ectoderm above and germ-wall below (see anterior end of Fig. 35). The first two regions seldom have greater widths than those in this series (cf. Figs 18 and 28), and hence the germ-wall is continually increasing in width. The subgerminal cavity is also increasing in diameter, but at a slower rate, and in this widening of the cavity there are left around its margin cells which were previously embedded in the yolk. These cells (Fig. XI, Y) constitute the under loose layer of the area opaca, and later enter into the formation of the yolk-sac entoderm, and, according to Ruckert, '06, also contribute to the vascular tissues. The inner edge of this lower layer becomes united to the free margin of the invaginated entoderm, when the latter spreads over the subgerminal cavity sufficiently to meet it. The first place for this union to occur is necessarily at the postero-lateral regions, and the last place is at the anterior end of the cavity.

Sections taken slightly to either side of the median line are of interest, in that they have vacuoles or cavities in the dorsal lip

(Figs. 38 and 39). The position of the cavities suggests that they are probably to be regarded as the remains of the cavity that was formed between the upper and lower layers when the former turned under to give rise to the latter. I might suggest that there is another possibility, namely, that such cavities are the homologue of "Kupffer's Vesicle."

Fig. 40, which is from a section taken through the plane *KF* of Fig. XI shows the tip of the right horn of the zone of junction

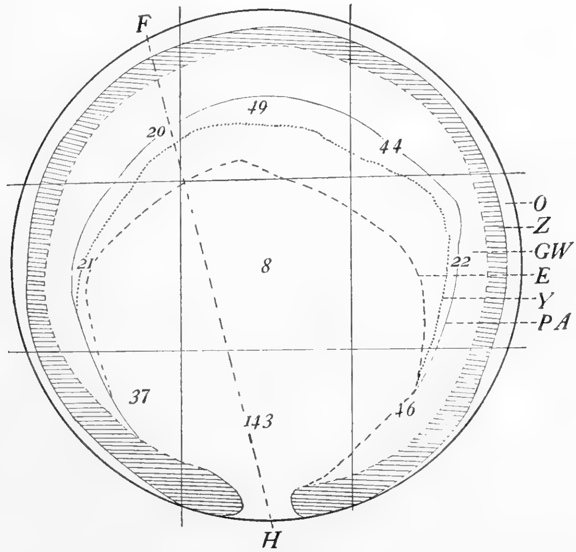

Fig. XII. Reconstruction of a blastoderm taken thirty-six and one-fourth hours after fertilization. Lettering is the same as in Fig. XI, *FH*, plane of section represented in Figs. 20 and 21. The numbers within the areas formed by the four intersecting lines indicate the number of degenerating periblastic nuclei in these areas. × 27.2.

and the lateral part of the dorsal lip. The length of the lip in section becomes less and less in passing laterally, finally disappearing altogether. Thus in Fig. 41 it is no longer present, and the margin is occupied by the zone of junction, inside of which is a region whose position would lead one to call it germ-wall, but it is probably more correct to regard it as a portion of the lip that has already been enclosed within the horns of the zone. Passing still farther to the

side one finds the posterior margin becoming less thick and gradually taking on the syncytial condition characteristic of the anterior and lateral parts of the zone of junction. However, it is not until one has reached about 45 degrees to either side of the median line that the posterior margin is found to be reduced to the average thickness of the rest of the edge.

Closing of the Blastopore.—It was stated above that the entoderm grows forward through the subgerminal cavity. The source from

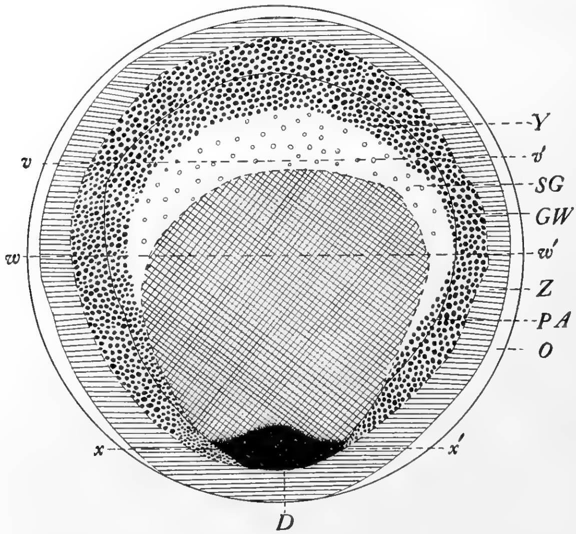

FIG. XIII. Reconstruction of a blastoderm taken thirty-five and one-fourth hours after fertilization. The blastopore has just closed, and the zone of junction completely encircles the blastoderm. *D*, is the enclosed dorsal lip. vv′, ww′, and xx′ are the planes of the sections illustrated in Figs. 24, 23, and 22 respectively. × 27.2.

which it draws its material for this forward growth is, of course, the thick dorsal lip. This results in producing either one of two conditions in the lip at the time the blastopore is closed. On the one hand, the entoderm may have grown forward to such an extent as to have produced a very perceptible diminution in the lip before the closing occurs. Such is the case in the blastoderm represented in Fig. XII, as is apparent from the median section (Figs. 20 and

On the other hand, in the majority of blastoderms there is no apparent reduction in the lip preceding the closing, but it remains quite as thick as that of Fig. 37, even after being entirely enclosed within the zone of junction. This can be made out from a series of transverse sections of the blastoderm shown in Fig. XIII. Thus in the section taken through xx′ the entoderm is at least five cells thick, and passes over gradually into the region of the zone of junction (Fig. 22). Above the entoderm a distinct ectoderm is differentiated, but slightly posterior to this it can no longer be distinguished, and still farther back is found the zone of junction, which completely encircles the blastoderm (Fig. XIII, Z). Anteriorly the entoderm rapidly thins out, the cells being arranged in groups (Fig. 23), which become less and less thick, finally disappearing altogether, so that only a few cells are found (Fig. 24).

Interruption of the Posterior Zone of Junction.—We are now in a position to consider the interruption of the zone of junction. It was stated above that the interruption was associated with the degeneration of the periblastic nuclei in the region of the posterior zone. Abundant evidence is found for this statement in the study of any egg taken either just before or during gastrulation. Thus in Figs. 8-13 is shown a series of nuclei in various stages of degeneration. The first indication of the breaking down process is found in the increase in the size of the nucleus. In this condition the nuclei do one of two things. In some cases, they stain intensely (Fig. 8) and apparently the nuclear membrane breaks down directly, leaving the chromatin lying free within the yolk (Fig. XIV, *A*). In the large majority of cases, however, they continue to increase in size and at the same time their capacity for stains gradually diminishes, until it is difficult to study them at all after the use of hæmatoxylin. When they have increased to a volume equal to many times that of an average normal nucleus, they begin to divide (rarely into equal parts—as in Fig. 10), or rather portions are pinched off from the sides of the nucleus (Figs. 9 and 11)—the process continuing until the entire nucleus is reduced to small fragments (Figs. 12 and 13). Finally one sees among the yolk spherules only clear spaces, which indicate the places previously occupied by these fragmenting nuclei.

Abnormal yolk or periblastic nuclei are found in many meroblastic eggs, especially those of the fish. Several of the nuclei figured by Raffæle, '98, for Belone, greatly resemble what I have observed in the Pigeon, and in the eggs of Squalus also are found many such nuclei. So far as I am aware, no one has described the complete fragmentation of the periblastic nuclei in the bird's egg, although Harper, '04, observed abnormal ones in early stages of the pigeon's egg. He regarded these as sperm nuclei, but in the light of Miss Blount's, '07, work, they are doubtless to be considered as periblastic nuclei, and are therefore undergoing this disintegrating process.

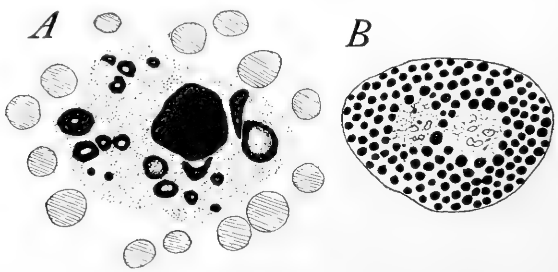

Fig. XIV. *A* is from the blastoderm shown in Fig. XII. It shows the chromatin lying free in a finely granular area among the yolk spherules—the nuclear membrane having disappeared. *B* is a yolk mass in which are two fragmenting nuclei. This mass had arisen from the yolk lying beneath the archenteron, and doubtless had taken up two degenerating nuclei which were in the central periblast. × 250..

The position of these nuclei within the egg is of importance; for in the main they are located in the region where the zone of junction is being interrupted. In the blastoderms illustrated in Figs. VI and VII they are found mainly in the yolk lying beneath the posterior margin—the rest of the edge being almost wholly free from them, in some cases entirely so. Later, when invagination begins, they are found around the greater portion of the margin (*e. g.,* in the series shown in Fig. VIII), and still later, they may be seen in all parts of the edge, but not in such abundance in the anterior half of the blastoderm as in the posterior, except during late gastrulation, when practically all of the nuclei beneath the archenteron have completely disappeared. In some few cases, however, even in late gastrulation,

there are many degenerating nuclei found in the yolk lying beneath the floor of the archenteron, but in such the nuclei are in the very last stages of disintegration (Fig. XIII). The absence of yolk nuclei beneath the archenteron is not characteristic of the birds alone, for in some of the Selachians also the same condition is found (*e. g.,* in Torpedo and Squalus).

In the interruption of the posterior zone of junction we have another line of comparison with the teleostean development; for this process is but the separation of the blastoderm from the underlying periblast. The comparison will become all the more obvious when we shall have shown experimentally that approximately that portion of the margin of the avian blastoderm, beneath which the zone of junction has disappeared, enters into the formation of the embryo. In other words, in the teleost the entire margin of the blastoderm separates from the periblast, and this whole margin (germ-ring) concresces to form the embryo; whereas, in the case of the bird, only about seventy to eighty degrees of the margin of the blastoderm parts company with the periblast, and just about this portion of the posterior edge is concerned in the process of concrescence.

Since abnormal nuclei are found as early as fifteen hours after fertilization (Harper, '04), there would seem to be some doubt regarding the possibility of such nuclei being instrumental in bringing about the interruption of the posterior zone of junction. Furthermore, I have found degenerating yolk nuclei in eggs taken several hours after gastrulation. Nevertheless, there is certainly no period, aside from that of gastrulation, in which they are in such abundance; and in addition to this, they are present mainly where the interruption takes place. The fact that such nuclei later are found gradually extending anteriorly around the margin, would only indicate that there was a tendency for the entire margin of the blastoderm to separate from the periblast.

c. Postgastrular Stages.

In eggs taken slightly later than the preceding, the entoderm is found not only to have grown farther forward, but also to have spread to the sides, so that its lateral margins have become united with the

inner edge of the germ-wall (Fig. XV). Hence, in transverse sections of the majority of blastoderms taken at this time, the entoderm will appear to be an outgrowth from the inner edge of the germ-wall. Fortunately, in not a few blastoderms the union between the invaginated entoderm and the germ-wall does not take place until about the time the egg is laid, and in such deferred cases it is easy to distinguish the lateral edge of the entoderm (Fig. 48), and thus to demonstrate that the gut-entoderm, at least in its lateral parts, does not receive elements from the germ-wall. Can the same be said

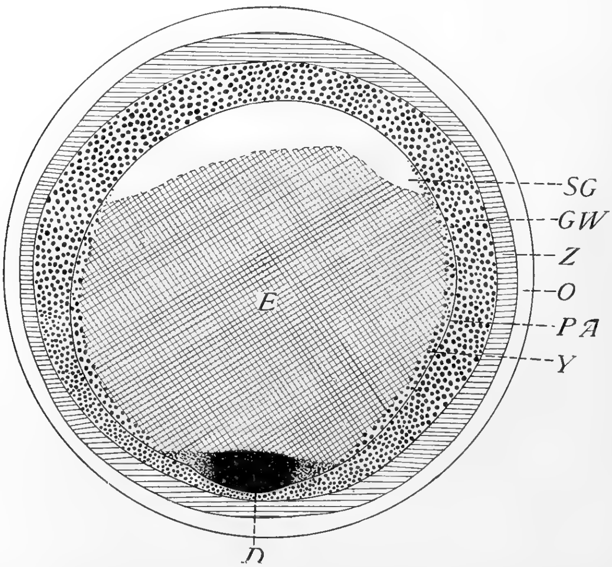

Fig. XV. Reconstruction of a blastoderm taken thirty-eight hours after fertilization. This blastoderm is approximately in the same stage of development as that of an unincubated hen's egg. $\times$ 27.2.

of its anterior and posterior parts? In regard to the former we can answer in the affirmative without hesitation; for in every blastoderm taken from the time of midgastrulation until three or four hours after incubation, there is found between the anterior limit of the entoderm and the germ-wall, a portion of the subgerminal cavity in which there are practically no nucleated cells. Indeed, in many blastoderms there are no cells, not even yolk masses (Fig.

45), and yet it is clear from measurements of such that the distance between the inner edge of the anterior germ-wall and the entoderm is growing less at the same time that the entoderm is increasing in length (Table III).

Concerning those cases in which cells (other than entoderm) and yolk masses are found in the cavity, more must be said. Many writers have described these elements in the chick blastoderm, and as far back as 1874 Goette figured them as arising from the yolk lying beneath the floor of the cavity, that is, from the central periblast. Recently Hertwig ('03) has described them, and in speaking of those that lie between the entoderm and the floor, he writes as

TABLE III.

Ser.	Age.	Length of Blastoderm.	Length of p. area.	Length of Entoderm.	Length of "Space." *
394	34 hrs.	2.190 mm.	1.547 mm.	0.119 mm.	1.428 mm.
342	34 "	2.261 "	1.666 "	0.595 "	1.071 "
254	36 "	2.667 "	2.071 "	1.476 "	0.595 "
256	37 "	2.976 "	2.262 "	1.786 "	0.476 "
332	38 "	3.190 "	2.215 "	1.786 "	0.429 "
377	40 "	3.219 "	2.310 "	1.905 "	0.405 "
341	44 "	4.048 "	2.238 "	2.143 "	0.095 "
195	46 "	4.809 "	2.619 "	2.619 "	0.000 "

* By the term space is meant the distance from the anterior limit of the entoderm to the inner edge of the exterior germ-wall.

follows: "Zwischen ihm und dem Dotterboden liegen in der Urdarmhöhle zerstreut einzelne kugelige Embryonalzellen, darunter auch grössere, dotterhaltige Kugeln, die Megasphären von His. Letztere haben nicht den Formwert einer Zelle, da Kerne auf keine Weise in ihnen sichtbar zu machen sind, wie von Gasser ('84) und anderen Beobachtern festgestellt worden ist. Sie sind daher nur vom darunter liegenden Dotter losgelöste, kugelige Ballen, die wohl allmählich zur Ernährung der Zellen der Keimblätter aufgebraucht werden. Auch im Raum zwischen den beiden Keimblättern kommen

wenige vereinzelte Zellen vor."[19] I fully concur with Hertwig's views regarding the fate of the non-nucleated yolk masses, for one can examine scarcely a series in which some evidence of their disintegration is not found. The manner in which these masses break up is of interest, in that the fragments often resemble cells. Thus, above and to the left of the mass at *dm*, Fig. 47, are smaller masses that have broken off and become spherical—a process probably comparable to the phenomena of surface tension. These smaller masses in turn continue to subdivide until the cavity may become crowded with very fine particles (Fig. 44) which in this state are doubtless taken up by the cells. The yolk masses therefore play no rôle in the formation of the primary germ layers, except, of course, indirectly as nutriment. The contention of Balfour ('73) that they may become nucleated by the formation of nuclei *de novo* from yolk spherules would scarcely accord with the views of modern cytologists.

Again, in regard to the significance of the nucleated elements within the cavity, I agree with Hertwig, who thinks that in numbers they are far too few to be of any importance in the formation of the germ layers. On making counts of these elements, I was surprised to find that in blastoderms such as shown in Fig. 47, less than two per cent of them are nucleated, and that even in this small number many of the nuclei show signs of degeneration (Fig. XIV, *B*). Not infrequently the cytoplasmic portion of such elements disintegrate, leaving the nucleus lying free within the cavity (Fig. 52, *n*). In other cases neither the cytoplasm nor nucleus breaks down at first, but the latter multiplies at the expense of the former until a solid mass of nuclei is formed (Fig. 55). Sooner or later these nuclei go to pieces.

These abnormal nuclei are to be accounted for by the fact that some of the yolk masses in arising from the central periblast (Fig. 46) naturally take up the periblastic nuclei, which, as was shown above, are degenerating. Their presence is in no way necessary to the formation of the masses, as is evident from the fact that the large majority of the masses are non-nucleated.

The phenomenon of yolk mass formation is only an index to the

[19] *Loc. cit.*, p. 858.

process of digestion, by which the blastoderm is securing its nourishment, and is doubtless similar to the phenomena of degeneration or fragmentation of the yolk that has been described by many workers on practically all of the vertebrate ova (Barfurth in the Teleosts; Dean in the Chimæroids; Stahl in the Reptiles; Ruge and Born in the Amphibians; Pflüger in the Mammals; Brunn and others in the Birds).

There are a few small cells within the cavity that are still to be accounted for (Fig. 53). These may come from two sources: either they are lower segmentation cells that have failed to get into the differentiating ectoderm, or they are wandering entoderm cells (Gasser, '82). If they come from the latter source and are later taken into the entoderm, no further consideration is necessary; but if they are to be regarded as coming from the former source, we may justly ask, Why is it that when an egg is fixed in an inverted position during the differentiation of the ectoderm, no cells are found in the cavity? Whatever be their source, they are too insignificant in numbers to be of any great importance.

So far, we have considered the question of whether or not the invaginated or gut-entoderm receives cells from the anterior or lateral parts of the germ-wall, and on the whole the evidence favors the negative; but in regard to the relation of the entoderm to the posterior germ-wall, further considerations are necessary. It was stated above that as a result of the manner in which the blastopore closes, the dorsal lip comes to lie within the margin of the blastoderm. Hence, in longitudinal sections, the entoderm, while ending anteriorly with a free margin (Fig. 42), appears to arise directly from the posterior germ-wall. This apparent union of the entoderm with the posterior wall is only secondary, and the greater part of the mass of cells here belongs to the dorsal lip. This is most obvious immediately after the closing of the blastopore, when the ectoderm is not differentiated from the underlying mass (Fig. 26).

Although Balfour ('82) and many other investigators, working on the unincubated hen's eggs, have noted that the entoderm is incomplete anteriorly and united to the germ-wall posteriorly, yet Nowack ('02) was the first to clearly state that the entoderm was

to be regarded as growing forward. He, however, not having studied the earlier stages, naturally supposed that the entoderm was an outgrowth from the posterior germ-wall, and thus missed the key to the origin of this germ-layer.

During the course of further development the entoderm completely penetrates the subgerminal cavity (Fig. XV, *SG*), and at the same time the mass of cells (lower cells of the dorsal lip) at its posterior border thins out to a single layer, thus showing that these cells contribute to the entoderm in its forward growth.

IV. Experimental Studies.

While many of the foregoing conclusions were first deduced from data gathered in a study of sections, yet they are of such a nature that experimental tests can be applied readily. Only a few of the many experiments that have been performed can be offered at this time, and these are selected, not because they are of any more interest than the others, but rather because they throw light on that mooted question, "How does the vertebrate embryo arise?" The two views that have been held by students of vertebrate embryology in regard to this question are too well known to need any discussion here. Both theories have been defended by able workers, but too often the attempt has been made to support the one to the exclusion of the other. This has been especially true of those who hold to the theory of differentiation.

The results obtained by experimental investigators have not been uniform. In the main, writers have been willing to admit that only a modified form of concrescence is found in the formation of the embryo. In the few desultory experiments (Assheton, '96, Kopsch, '02) that have been made on the chick blastoderm only negative results have been found. This failure to secure positive evidence is due to two causes. In the first place, the technique has not been sufficiently refined. Thus, Assheton used sable hairs which he inserted in the unincubated blastoderm on either side of the axial line on the boundary between the areas opaca and pellucida. The results were negative, as one might expect; for who would suppose that the force exerted by the movement of materials in the delicate

blastoderm could be sufficient to overcome the resistance offered by the hair held above by the vitelline membrane and below by the yolk, even, indeed, if such material did not merely flow around the obstructing hair. In the second place, both Kopsch and Assheton were operating at a time when concrescence either had ceased altogether, or its influence was waning. Thus in Kopsch's ('02) experiment VII the operation was made after twelve hours of incubation, at a time when the bulk of the axial material had been marshalled from a lateral into a median position for a period of at least twelve to fifteen hours. It is obvious, from the above morphological data, that any experiments from which we could hope to gain any insight into the part played by concrescence, must be made during gastrulation, for concrescence and gastrulation are but different phases of the same process.

If the avian embryo is the product of concrescence and the right and left parts of the dorsal lip represent the homotypical halves of the future embryo, then injuries made on the posterior margin at different distances from the median line during early gastrulation, ought later to appear at different levels in the embryo, that is, an injury made at 10° from the median line ought to appear nearer the head region than one made at 45°. Furthermore, such injuries ought to affect only that half of the embryo which corresponds to the side of the dorsal lip injured. The progress of concrescence can be tested by operating on successively older stages. Thus, the following sets of operations will be described: Set A, on early gastrular stages; Set B, on late gastrular stages; and Set C, on unincubated and early incubation stages.

Set A—On Early Gastrular Stages.

Experiment III.

An injury made in the middle of the dorsal lip slightly within the margin (Fig. XVI, *a*) is later found in the cephalic region of the embryo, greatly disturbing the material of what is later to become the primary fore-brain (Fig. 59, *op*). While the section through this injured region shows the affected cells to be situated slightly to the right of the median line, yet the entire head-fold is disturbed

(Fig. 57). The result of such an operation leaves but two alternatives with reference to the position of the embryonic primordium at the time when the injury was made. Either we must suppose that this primordium was situated in the exceedingly small space between the operated region and the posterior margin (Fig. XVI, *a*), or that its right and left halves lay along the lateral margins, and were gradually brought together by concrescence. That the latter alternative is the correct one will become obvious from the results of the following experiments.

Experiment IV.

In this experiment the operation was made ten degrees to the right of the median line, the needle being set so that the outer edge

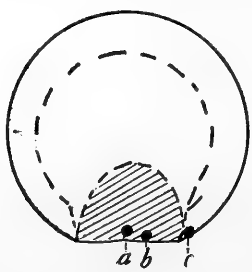

Fig. XVI. Scheme for operating in Experiments III, IV, and V.

of the resulting injury coincided with the margin of the blastoderm (Fig. XVI, *b*). After thirty-six and three-fourths hours of incubation the injury was found on the right neural fold in the mid-brain region (Fig. 63). Although the left neural fold is slightly distorted, yet the section shows with great clearness that the affected cells are found only on the right side (Fig. 62). All the structures characteristic of this region in a normal embryo, are here found well developed. As we should expect, the mesoderm and chorda are uninjured, for when the operation was performed these structures were not yet present in the head region.

Experiment V.

Passing now to the experiment in which the operation was made forty-five degrees to the right of the middle line (Fig. XVI, *c*), we

find that the injury is situated seven sections anterior to the posterior end of the resulting embryo (Fig. 71 *op*), that is, the injured cells have been moved from a lateral into a median position. In the sketch of the transverse section through the affected region (Fig. XVII) the mass of cells is seen to be located slightly to the right side. While the needle destroyed a considerable portion of the primitive streak material, yet the blastoderm has apparently recovered from the injury, with the mass of affected cells separated from the blastoderm proper.

The apparent recovery of the blastoderm from the operation is to be explained by the fact that the injury, being made so far to the side, affected a region less highly differentiated than in the case of the operation at ten degrees. That is what one would expect,

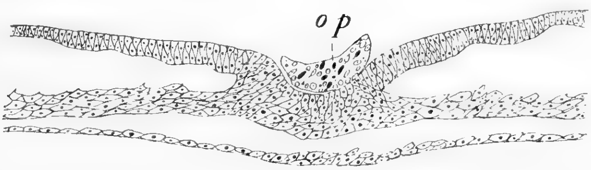

Fig. XVII. Transverse section through the injured region of the embryo shown in Fig. 71. See text for description. × 95.

for just as at any given period of the early development, the anterior portion of the embryo (or primordium) is in a higher state of differentiation than the posterior.

The results of the above experiments (III-V) show very clearly that the axial portion of the embryo arises from material previously situated in the right and left halves of the dorsal lip, material brought together by the process of concrescence. These experiments have been repeated several times, both in the manner described above, and with certain variations. Thus an operation made on the lip at twenty degrees either to the right or to the left of the axial line, is later found at the level of the anterior somites. In this paper we are not concerned, however, in analyzing the exact morphological value of the different parts of the blastoporic lip, but rather in showing that the avian embryo is the product of concrescence.

If the theory of concrescence is correct, it is obvious from these

experiments that similar operations made at a later period of development should be found located more posteriorly in the resulting embryo. Thus in a late gastrulation stage, an injury made in the middle of the dorsal lip just anterior to the posterior margin, should be found later not in the fore-brain, as in Experiment III, but at a point situated more posteriorly to that region. Furthermore, if an injury be made on the margin to the side of the axial line at such a stage, it ought later to appear in the corresponding side toward the posterior end of the embryo. The results of such operations are shown in the following set of experiments.

Set B. On Late Gastrular Stages.

Experiment VI.

The scheme for this operation appears in Fig. XVIII, *a*. It would be equivalent to injuring the posterior margin of such a blastoderm as that shown in Fig. V, *J*. The result of the operation is shown in Fig. 54, in which the injury is seen to lie at the level

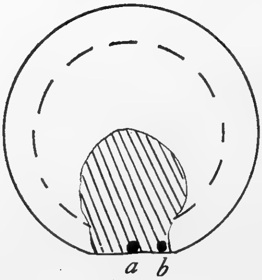

Fig. XVIII. Scheme for operating in Experiments VI, VII and VIII.

of the tenth pair of somites. Posterior to the affected region there is found a normal primitive streak, which has not yet differentiated into the posterior axial portion of the embryo. The transverse section (Fig. XIX) shows a mass of dead cells lying between the separated halves of the neural tube, and a few scattered dead cells lying just above the entoderm, which is intact. The notochord is situated on the right side.

It would seem from this result that although the halves of the dorsal lip have been unable to coalesce in the region of the injury,

yet they are capable of giving rise to the normal structures characteristic of this region. Posterior to the injury, however, they have succeeded in fusing and forming the primitive streak material.

Experiment VII.

If an operation be made similar to the preceding, but at a slightly later period, it is not found in the body of the embryo, but at the extreme posterior end (Fig. 64, *op*).

Experiment VIII.

In this experiment the injury was made twenty degrees to the right of the axial line (Fig. XVIII, *b*) at thirty-six hours after fertilization, that is, at a stage corresponding to the one shown in Fig. V. *J*. The egg was then incubated for forty-eight hours. The injury is

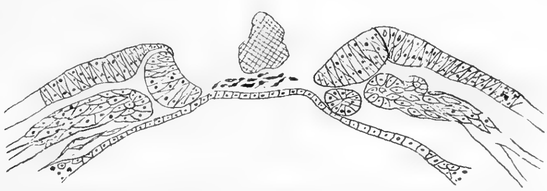

FIG. XIX. Transverse section through the injured region of the embryo shown in Fig 54. $\times$ 95.

located on the right neural fold, at about one-third the distance from the posterior end to the first mesoblastic somite (Fig. XX, *op*). The left neural fold is uninjured. The result of such an experiment admits certainly of no other explanation than that the mass of affected cells has moved from a marginal into an axial position.

SET C. ON UNINCUBATED AND EARLY INCUBATED STAGES.

In this set of experiments I shall endeavor to show that not all of the embryonic material has been brought into a median or axial position at the time when the egg is laid; but that it lies to either side, on the boundary between the areas opaca and pellucida. The presence of a slightly more opaque spot in this region has already been noted in connection with the study of surface views (Fig. V,*K*),

as well as in the study of sections (Fig. 43.) Furthermore, Koller ('79 and '81) has figured and described for the unincubated chick blastoderm a thickening in this region.

Experiment IX.

If a very small injury be made on the boundary between the two areas in line with the axis of the future embryo (Fig. XXI,*b*), it is

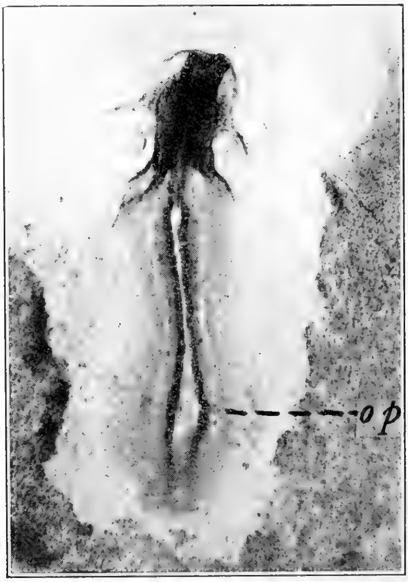

Fig. XX. A photograph of the embryo described in Experiment VIII. The injured cells are seen at *op* on the right neural fold in the region of the open myelon. The primitive streak is bifurcated at the posterior end.

found later some distance from the posterior end of the embryo (Fig. 60, *op*). Such an operation destroys a considerable portion of the primitive streak material in the region over which it extends (Fig. 58), but for twenty-five sections posterior to the injury a normal primitive streak is found. The point of interest in this experiment lies in the question regarding the source of the material from which the tail of the embryo is developed. The material must lie just posterior to the pellucid area, or to either side of the axial line on

the boundary between the two areas. If from the former source, the material would be disturbed by an injury made in the area opaca just posterior to the pellucid area; but if from the latter source, it would be affected only by operations made to the side of the axial line on the boundary between the two areas.

Experiment X.

The operation was made just posterior to the pellucid area (Fig. XXI,*a*). The injury has in no way affected the embryo (Fig. 56), but lies posterior to it in the area opaca. Assheton ('96) has performed a similar experiment on the chick blastoderm, using a sable hair instead of the needle. He also found the embryo uninjured. From the result of this operation it is evident that the material out of which the tail of the embryo is differentiated does not lie just posterior to the pellucid area.

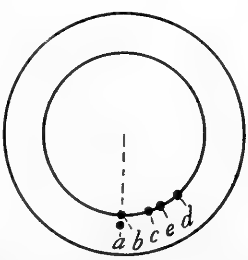

FIG. XXI. Scheme for operating in Experiments IX-XIII.

Experiment XI.

If the injury be made on the boundary between the two areas twenty degrees to the right of the middle line (Fig. XXI,*c*), it is found later drawn into the side of the embryo (Fig. 68) a short distance from the posterior end. In the transverse section through the operated region, it is seen that just half of the axial material is affected (Fig. 61). This is of the greatest importance, because it shows that the tail end of the embryo is formed by the concrescence of material lying to either side of the middle line. The limit to which material extends laterally is shown in the following experiments.

Experiment XII.

This experiment is similar to the preceding, except that the operation was made 30 degrees to the right instead of twenty (Fig. XXI,*e*), and in the resulting embryo the injury is found in the right side of the posterior end of the primitive streak (Fig. 69).

Experiment XIII.

An injury made still farther laterally (Fig. XXI,*d*) does not affect the embryo (Fig. 70), but is found in relatively the same position as that in which it was performed.

The results obtained from this set of experiments can leave no doubt concerning the presence of portions of the dorsal lip which lie between the boundary of the two areas, and which have not yet completely fused together at the time when the egg is laid. The manner by which this region is established has been considered in connection with an earlier stage (Fig. XV). It is this structure doubtless that Koller ('79) has described for the unincubated chick blastoderm, and which is often called *Koller's crescent.* In the pigeon I have never observed a "crescentric groove" as described by Koller,[20] and furthermore this region does not give rise to the entire primitive streak, but only to the posterior part. Even then it usually completely disappears three or four hours before the primitive streak becomes visible.

V. Discussion and Summary.

Discussion.

Throughout the foregoing pages the term gastrulation is employed to designate the process of invagination by which the gut-entoderm takes its rise, together with the concomitant phenomenon of concrescence. It may therefore seem to be used in a sense that does not

[20]I refer here to the presence of a groove during the early hours of incubation. It is true that in later stages (*e. g.*, Fig. 20) one sometimes finds the posterior end of the primitive streak bifurcated, but these must be regarded as much delayed cases, and are similar to those figured by Schauinsland ('03) for the sparrow.

accord with general usages; as, *e. g.,* in the case of the lower vertebrates, Amphioxus and the fishes, where the gut-entoderm, mesoderm, and chorda are all said to be involuted at the same time in the form of the primary entoderm.

This objection, however, completely disappears if the primitive streak formation is regarded as a part of the gastrular phenomenon. That such an explanation for the primitive streak formation is fully justified is obvious when considered in the light of comparative embryology. Thus in Amphioxus all of the chorda and mesoderm are derived from the primary invaginated layer. In Amphibians the posterior part of the mesoblast is formed about the lips of the blastopore, and is often spoken of as the "peristomal mesoblast," in contrast to the more anterior portion, or "gastral" mesoblast. In this case, no one hesitates to consider the whole process in Amphibians as that of gastrulation, because the "gastral" and "peristomal" mesoblast are directly continuous with one another. In the case of birds, all of the mesoblast is derived from the primitive streak, that is, it is all peristomal mesoblast. In the bird, therefore, the transition from a gastral to a peristomal mesoblastic formation has gone a step farther than in the case of the Amphibians. We have shown experimentally that as the gut-entoderm is being involuted, the concrescence of the halves of the dorsal lip is also taking place. Furthermore, there arises later from this fused region the primitive streak, or mesoblast. It is evident, therefore, that the invagination of the gut-entoderm and the primitive streak formation are but different parts of the same process, namely, that of gastrulation. The occurrence of a short period (from shortly after the closing of the blastopore to the appearance of the primitive streak), during which one cannot distinguish, either by sections or by surface views, the primitive region, in no wise invalidates the above comparison.

Hertwig ('03) has divided the process of gastrulation in the amniota into two parts or phases. He, however, had little else to offer for his first phase in the bird other than Duval's work—a work over which he himself casts a shadow of doubt as to correctness, as is evident from the citation given in the first part of this paper.

It is not my purpose to enter into a discussion of the whole ques-

tion of concrescence, for several able papers dealing with the problem have appeared from time to time since His first clearly stated the theory (Semper, '76; Whitman, '78 and '83; Rauber, '76; Kollmann, '85; Ryder, '85; Minot, '90, and others). It is sufficient to state here my conclusion, that concrescence is the method by which the avian embryo takes its rise. The conclusion is supported not only by experimental evidence, but also by the structure of such blastoderms as shown in Fig. V,*L*, as well as by that of the rare ones (Whitman, '83). In fact, it matters not from what angle we approach the problem, the conclusion is the same, namely, that the axial material of the avian embryo is derived from the fused lateral parts of the blastoporic lip.

The anterior limit to which concrescence is operative in the formation of the avian embryo is another problem, but it would seem, from the result of Experiment IV, that at least that portion of the embryo which lies posterior to the primary fore-brain is formed by concrescence. This is in accord with the experimental results of Peebles ('04) and Kopsch ('02); especially those of the latter, who maintains that all of the embryo except the pre-chordal head area arises directly from the primitive streak material (that is, from material that is formed by concrescence).

In this paper I have endeavored to establish two main points with reference to avian development: (1) that the gut-entoderm is formed by invagination; (2) that concrescence is the method of embryo formation. If I have been successful in establishing these two points, it follows that the early development of the birds can be brought into complete harmony with that of other vertebrates; for although differences do exist, yet they are those for which comparative embryology has an explanation. Indeed, in the avian development the differences have been brought about very largely as a result of the enormous accumulation of yolk within the ovum. Even concrescence itself has been made necessary as a result of this accumulation, and for that reason it is a process that is to be regarded as coenogenetic rather than as palingenetic. If concrescence is considered as a secondary process, we ought not to expect to find it in the embryo formation of those vertebrates that have ova practically

wanting in yolk; and as matter of fact a majority of the investigators on the development of Amphioxus maintain that there is no concrescence, as does Conklin ('05) also for the Ascidians. Eycleshymer ('02), as a result of his experimental studies on the Amphibian egg, concludes also that concrescence is a secondary process. He says, "that in those Amphibia which approach most nearly the holoblastic type, as Rana, Bufo, Acris, and Chorophilus, the greater portion of the embryo is formed through differentiation *in situ* and overgrowth, concrescence being confined to a limited region at the caudal end of the embryo. In those forms like Necturus in which there is a marked meroblastic tendency, due to the relative increase in the amount of yolk, a lesser extent of the embryo is formed through differentiation *in situ,* while there is a corresponding increase in the extent of the embryo formed through concrescence, or coalescence of the lateral margins of the blastopore." Again, in his concluding paragraph he writes that "there is every reason for maintaining that differentiation *in situ* is the primitive method of embryo formation, concrescence being a secondary process which has progressed *pari passu* with the increase of yolk material."[21]

Owing to the close affinities existing between birds and reptiles, we should expect to find many points of comparison in their modes of development. Although many writers have pointed out the similarities existing between the two modes, yet, judging from the results obtained in the study of the pigeon, it would be of the greatest interest to be able to trace the origin of the "Primitive Plate of Will" to the margin of the blastoderm, and thus to establish a more exact comparison between the two forms.

Summary.

The main points brought out in this paper may be stated in the following brief summary:

1. Gastrulation in the pigeon's egg is preceded by the thinning out of the thickened blastodisc. The thinning-out process begins at about twenty-one hours after fertilization, and consists in the crowd-

[21] *Loc. cit.*, p. 353.

ing upward of the lower segmentation cells in between the superficial ones, finally reducing the entire central region to a single layer—the primary ectoderm. The thinning-out begins slightly posterior to the center of the disc and then spreads in all directions, but with more rapidity toward the posterior margin. The thinned-out central region is the beginning of the *area pellucida.*

2. Between thirty and thirty-three hours after fertilization the *zone of junction,* or the region where the marginal cells are open to the white yolk or periblast, becomes interrupted for a distance of seventy to eighty degrees at the posterior margin. Hence, this margin of the blastoderm now ends with a free edge. The interruption is but the separation of the blastodisc from the underlying periblast, and has associated with it the degeneration of periblastic nuclei.

3. At about thirty-four hours after fertilization (or seven hours before the egg is laid) there occurs the gastrula-invagination. This consists in the rolling under of the free posterior edge of the blastoderm, together with the simultaneous forward growth of the involuted cells. The invaginated cells are arranged in the form of a tongue-like process, which finally penetrates the subgerminal cavity (enlarged segmentation cavity). It does not reach the anterior limit of this cavity until from three to four hours after the beginning of incubation.

4. Immediately after the gastrula-invagination occurs, the rounded posterior margin thickens up; in part by the multiplication of the cells *in situ,* but mainly by the movement of material from the right and left halves of the dorsal lip, which come together and coalesce in the middle line—a process to be regarded as a form of "concrescence."

5. The median region formed by the coalescence of the lips of the blastopore is the primordium out of which the primitive streak develops. Since the primitive streak gives rise to the mesoderm and chorda, its formation is to be considered as a part of gastrulation.

UNIVERSITY OF CHICAGO, JUNE, 1908.

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COMMON REFERENCE LETTERS USED IN THE FIGURES.

a, anterior end of the blastoderm.
ac, archenteric cavity.
b, blastopore.
cc, individualizing cells.
d, dorsal lip of the blastopore.
e, invaginated or gut-entoderm.
E, region covered by gut-entoderm.
ec, ectoderm.
gw, germ-wall.
m, yolk masses.
np, Nucleus of Pander.
o, region of overgrowth.
p, posterior end of the blastoderm.
pn, periblastic nucleus.
s, segmentation cells.
sc, segmentation cavity.
sg, subgerminal cavity.
z, zone of junction.

DESCRIPTION OF FIGURES.

PLATE I.

FIG. 1. A median longitudinal section of a blastoderm taken twenty-one hours after fertilization, or twenty hours before laying. × 117.

FIG. 2. A portion of the anterior half of a median longitudinal section from a blastoderm taken about thirty-three hours after fertilization, or eight hours before laying. It is not so far advanced in its development as blastoderms usually are when taken at this time. See text for description. × 161. (See also Figs. 29 and VI).

FIG. 3. A portion of the posterior half of the same section as preceding. Compare these two figures as to the condition of the zone of junction. × 161.

FIG. 4. Posterior end of a section, twenty sections to the right of the preceding. Note especially the cells organizing about the periblastic nuclei at "*cc*," and also the two degenerating periblastic nuclei (*pn*). × 365.

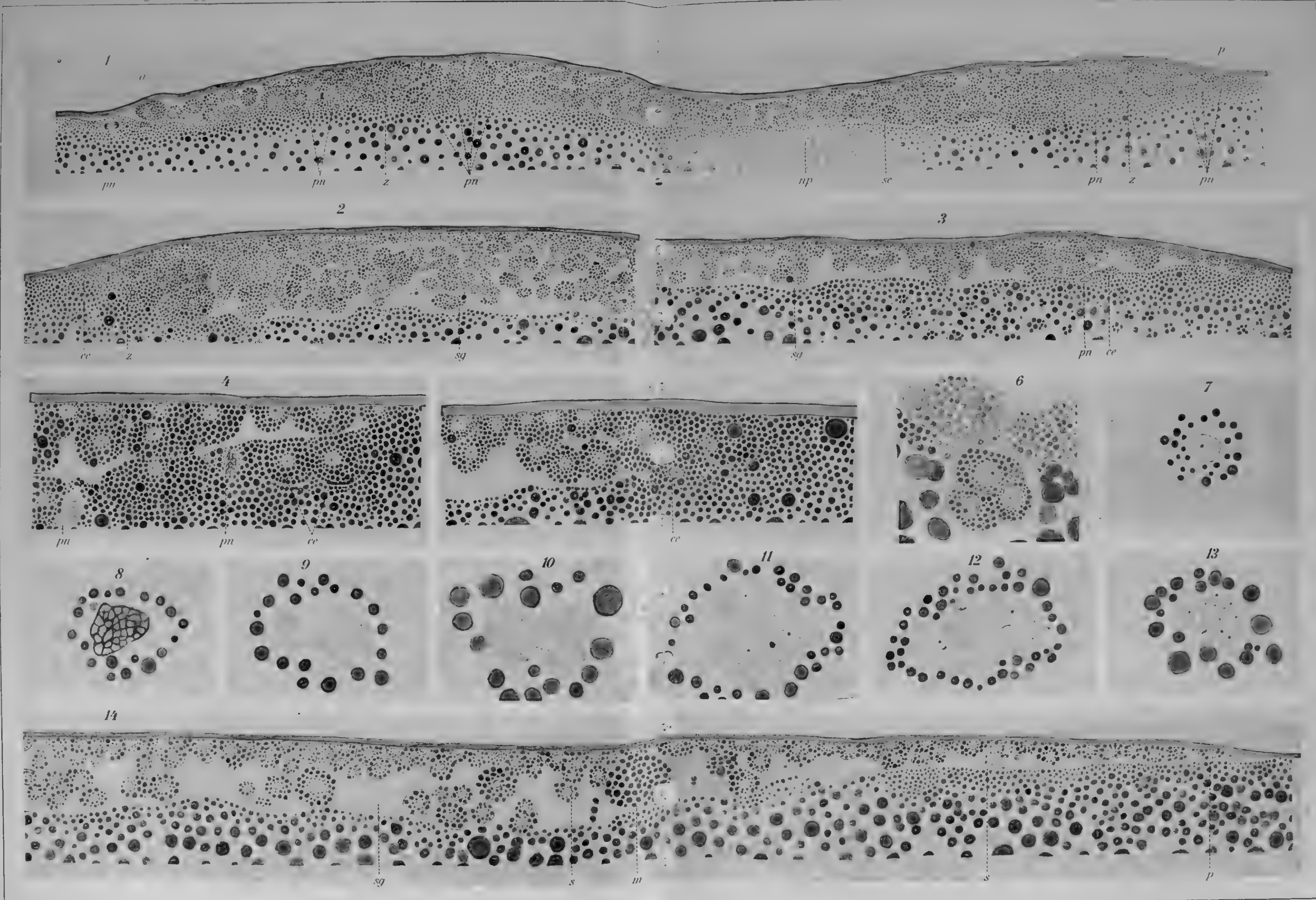
FIG. 5. Posterior end of a section, five sections to the right of the preceding. This shows the tip of the right horn of the zone of junction. × 365 (see also Fig. VI).

FIG. 6. A group of six (three shown in the section) cells organized [illegible] periblastic nuclei, which are in the central periblast. × 657.

FIG. 7. A normal periblastic nucleus which was introduced for comparison with the following figures (8-13). × 886.

FIGS. 8-13. Various stages of degenerating periblastic nuclei. × 886.

FIG. 14. Posterior end of a longitudinal section taken slightly to the left of the median line. It shows the thin epithelial-like margin just before invagination occurs. At "*m*" is one of the few yolk masses that are found at this stage. × 259. (See also Figs. 30 and VII).



Werner & Winter, Frankfurt a/M.

Plate II.

Fig. 15. Posterior end of an oblique section from a blastoderm taken thirty-four hours after fertilization, or seven hours before laying. The yolk is cracked and as a result granules are found in the cavity (*c*) just posterior to the dorsal-lip. See text for description, and also Fig. VIII. × 339.

Fig. 16.—Posterior end of a section taken through y-y′, Fig. VIII. The section ends with a thin free margin. × 259.

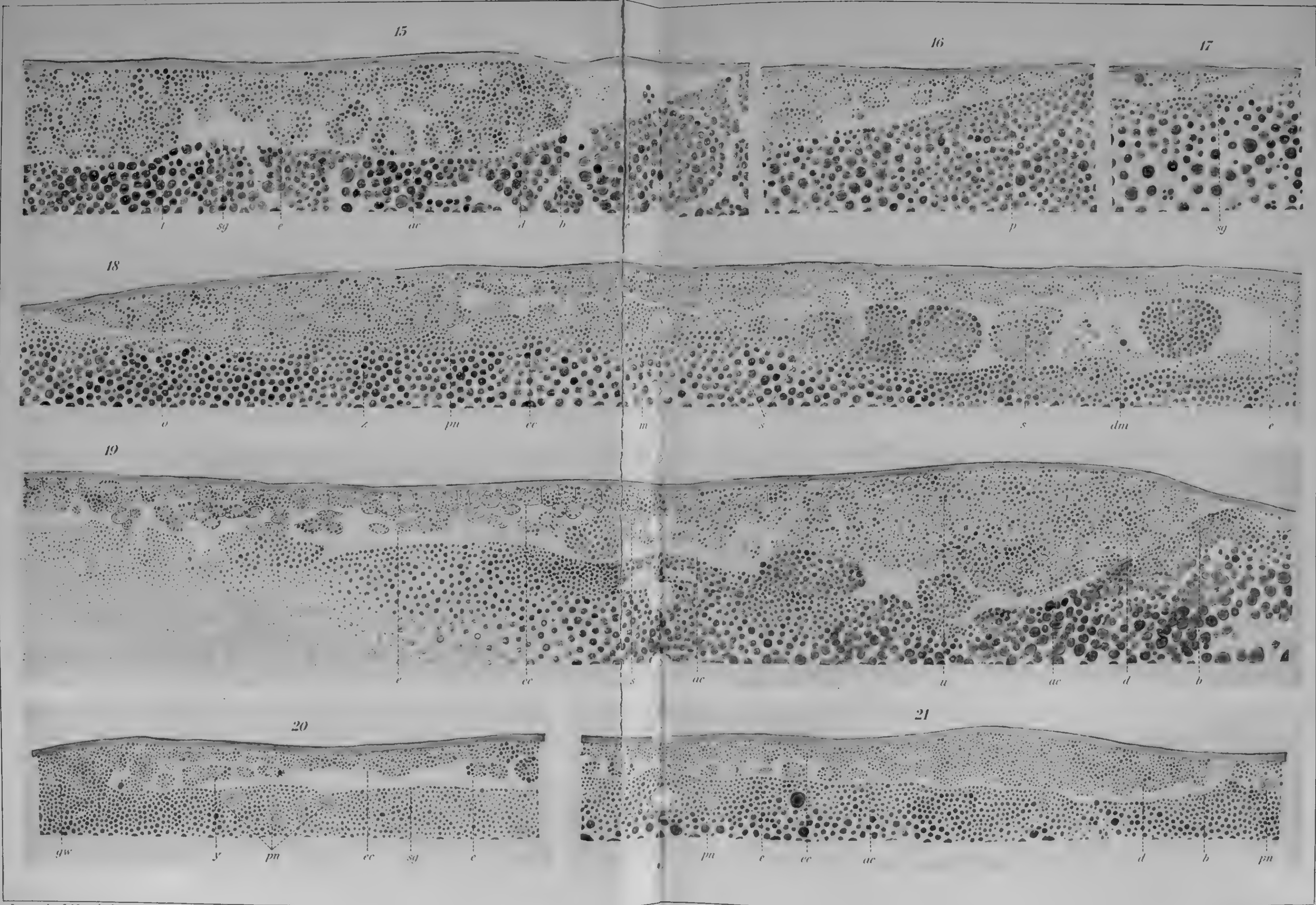
Fig. 17. Posterior end of a section slightly to the right of the one represented in Fig. 14. It shows how a considerable cavity may develop beneath the margin before invagination begins. × 259.

Fig. 18. Anterior third of a section taken in the plane passing through *CR* of Fig. XI. The thinning-out is in the last stages, and at the points marked "s" a few cells have loosened and sunk down. Otherwise the subgerminal cavity contains no nucleated cells—only large yolk masses are present, some of which are disintegrating (*dm*). See text for description, and also Figs. 35 and 36. × 259.

Fig. 19. Posterior third of the same section as preceding. *u*, union between the deeper cells of the dorsal-lip and the entoderm. See text and Figs. 35 and 37 for description. × 259.

Fig. 20. Anterior part of a section taken in the plane passing through *FH* of Fig. XII. Three large degenerating periblastic nuclei are shown (*pn*), and at "y" are the cells which constitute the inner margin of the germ-wall. The zone of junction is too far to the left to be seen in the figure. × 259.

Fig. 21. Posterior part of the preceding section. See text. × 259.



Werner & Winter, Frankfurt a/M.

Plate III.

Fig. 22. Right half of a transverse section through the plane xx' of Fig. XIII. $\times$ 259.

Fig. 23. A portion of the right side of a section passing through ww' of Fig. XIII. $\times$ 259.

Fig. 24. A portion of the central part of a section passing through vv' of Fig. XIII. $\times$ 259.

Fig. 25. A part of the region of overgrowth from the series which is reconstructed in Fig. XI. It shows a large periblastic nucleus which has moved down from the edge of the blastoderm. This is the only periblastic nucleus in the series that was found either beneath or external to the region of overgrowth. $\times$ 657.

Fig. 26. Posterior portion of a median longitudinal section from a blastoderm taken thirty-seven hours after fertilization, or four hours before laying. At "*d*" is shown the dorsal-lip of the blastopore, which has been enclosed within the zone of junction. It is doubtful whether the region marked "*gw*" should be regarded as germ-wall. $\times$ 152.

Fig. 27. Left side of a median transverse section from a blastoderm taken about thirty-five hours after fertilization, or six hours before laying. It shows the beginning of the region of overgrowth at "*o*." $\times$ 138.

Fig. 28. Posterior end of a longitudinal section from a blastoderm taken four hours after incubation. Introduced to show the condition of the region of overgrowth at this time. $\times$ 138.

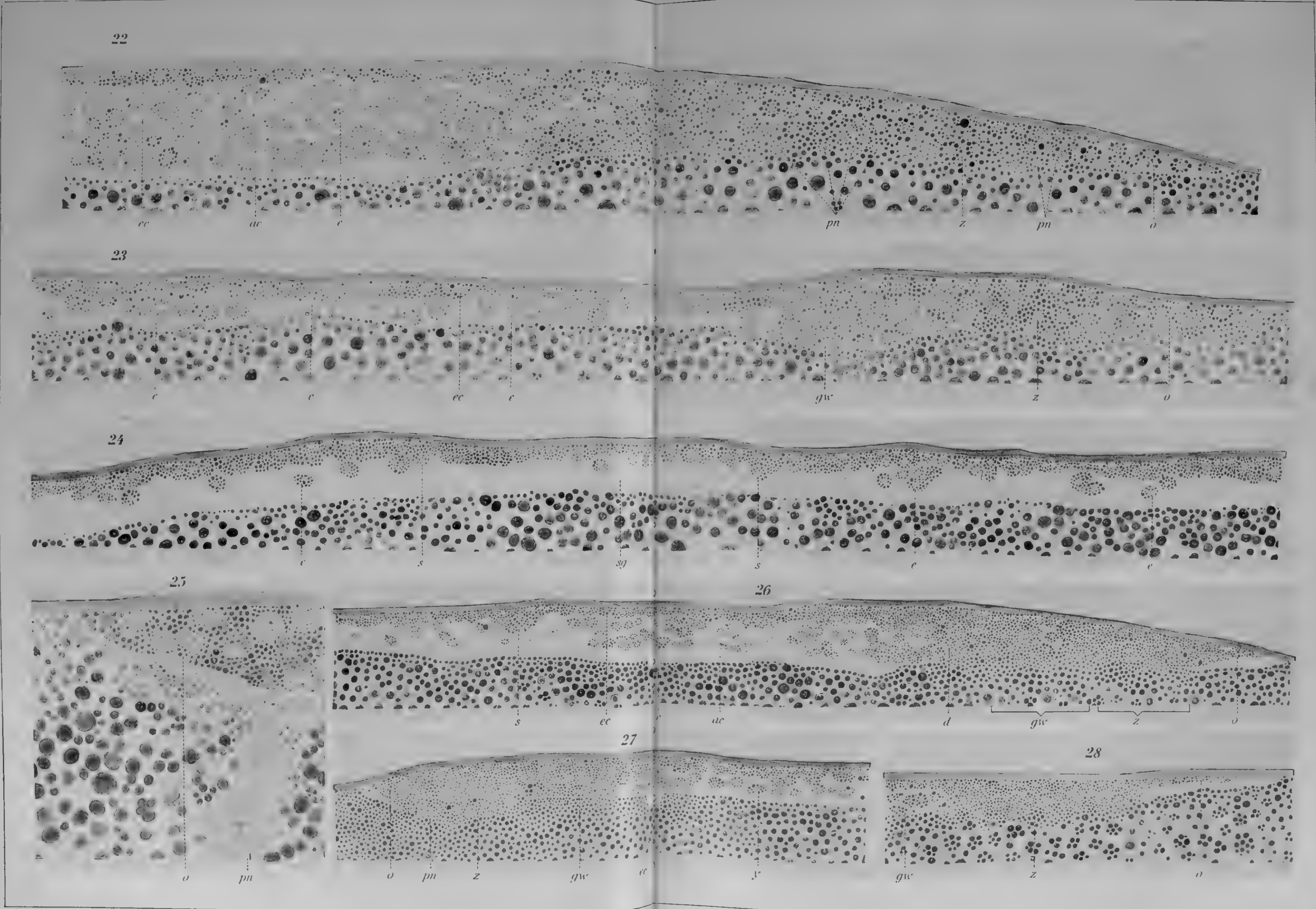

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Plate IV.

All the photographs in Plates IV-VI are from the sections of the blastoderms, and the prints were made directly from these negatives without any retouching. The Zeiss apo., 8 and 16 mm. lenses, and compensating ocular 4 were used with camera draw varying from 12 to 20 inches. The magnification is given in each case.

All of the photographs in Plates VII-X were made directly either from the whole mount preparations or from the sections, with various combinations of lenses. In each case the magnification is given.

Fig. 29. A median longitudinal section taken through *CR* of Fig. VI. Note especially the gradual increase in the depth of the blastoderm in passing from the right (posterior) to left (anterior). $\times$ 143.

Fig. 30. Longitudinal section taken slightly to the left of the line *CD* of Fig. VII. The thinning-out is farther advanced than in the preceding section, and at the point marked "x" is clearly shown the cells that have loosened and sunk down during fixation. Otherwise the subgerminal cavity would contain only a few non-nucleated yolk masses. $\times$ 117.

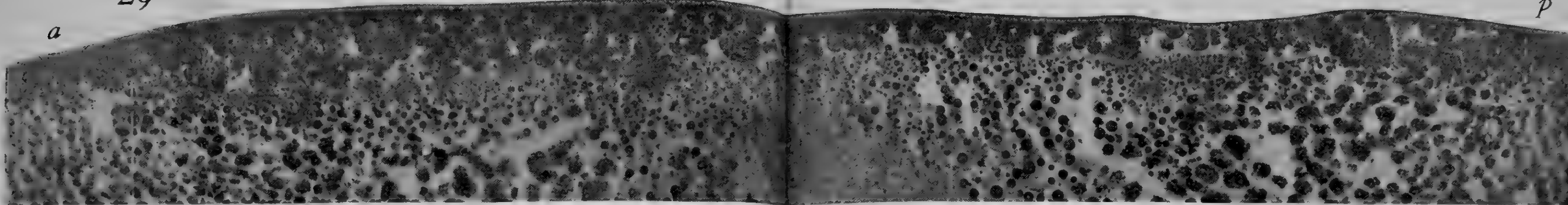
Fig. 31. An enlarged portion of the posterior end of the preceding photograph. $\times$ 301.

Fig. 32. Posterior end of a section taken through plane x-x′ of Fig. VIII. See text for description. $\times$ 366.

Fig. 33. Posterior end of a longitudinal section, seven sections to the left of the median line, from a blastoderm taken thirty-four hours after fertilization, or seven hours before laying. The anterior limit of the entoderm is shown at "*e*." $\times$ 193.

Fig. 34. The median section of the same blastoderm as the preceding. The length of the invaginated entoderm is necessarily greater than in Fig. 33. $\times$ 193.

29



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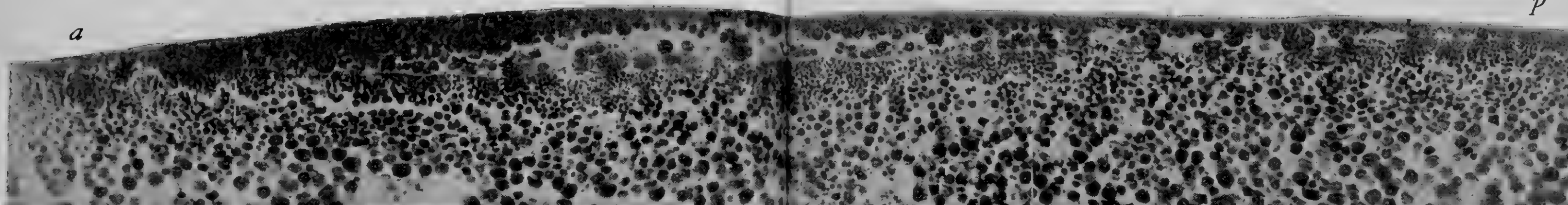

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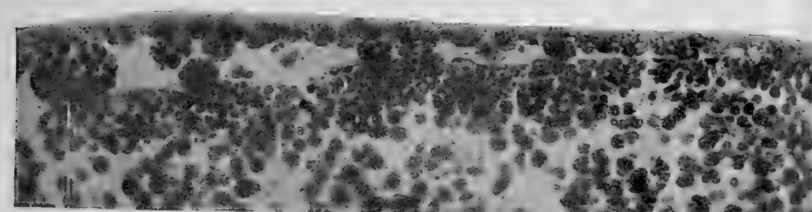

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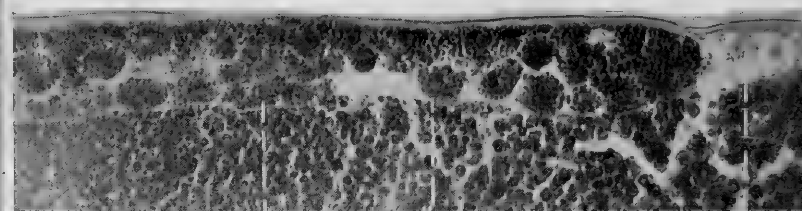

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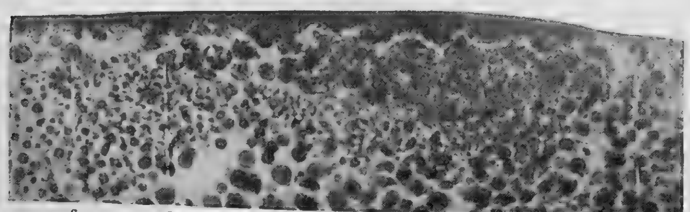

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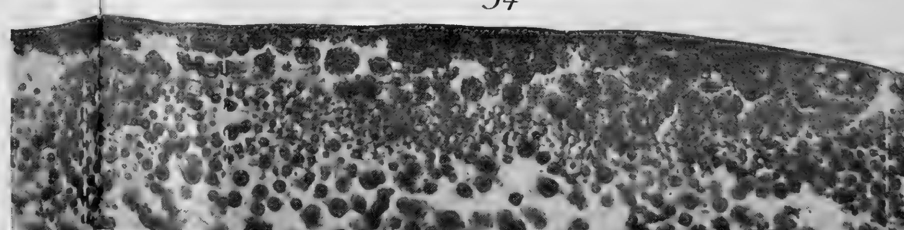


PLATE V.

FIG. 35. A median longitudinal section taken in the plane *CR* of Fig. XI. See text for description. $\times$ 107.

FIG. 36. Enlarged anterior end of the preceding. $\times$ 245.

FIG. 37. Enlarged posterior end of Fig. 35. $\times$ 245.

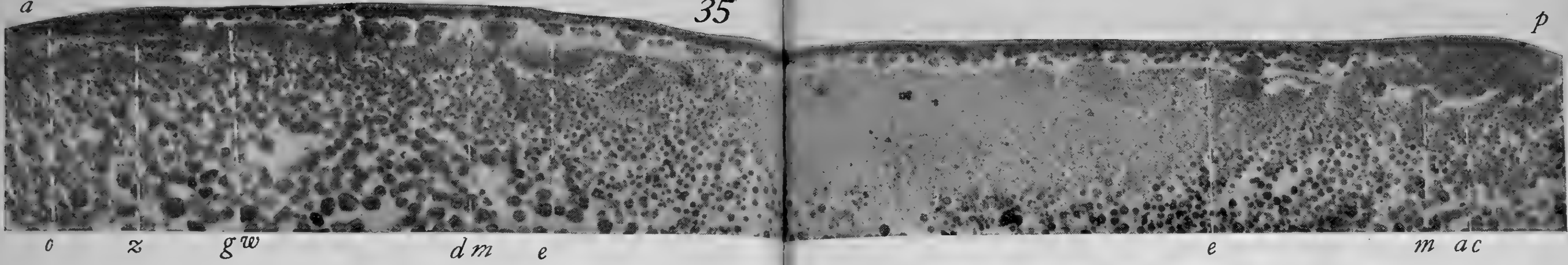
FIG. 38. From a section, four sections to the left of the one represented in Fig. 35. The cavity in the thick dorsal lip is, perhaps, the remains of the space that was formed between the upper and lower layers when the former turned under to give rise to the latter. $\times$ 245.

FIG. 39. From a section, two sections to the right of the one represented in Fig. 35. It shows the same conditions as the preceding. $\times$ 245.

FIG. 40. Posterior end of a section taken through *KF* of Fig. XI. The tip end of the zone of junction (z) is shown, and also the lateral portion of the dorsal lip of the blastopore. $\times$ 245.

FIG. 41. Posterior end of a section taken through *GH* of Fig XI. There is no overhanging margin (dorsal lip) in this section. $\times$ 245.

35



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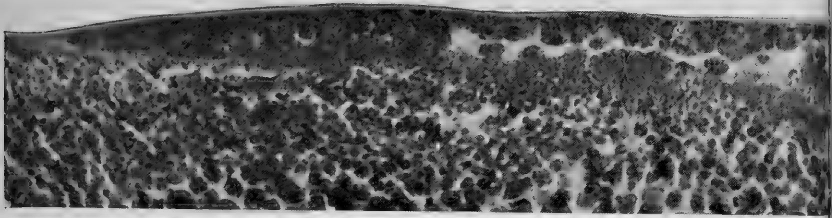

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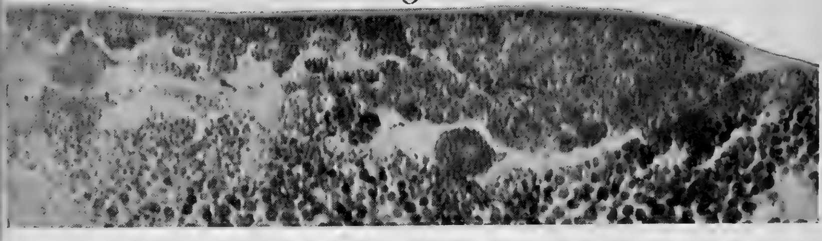

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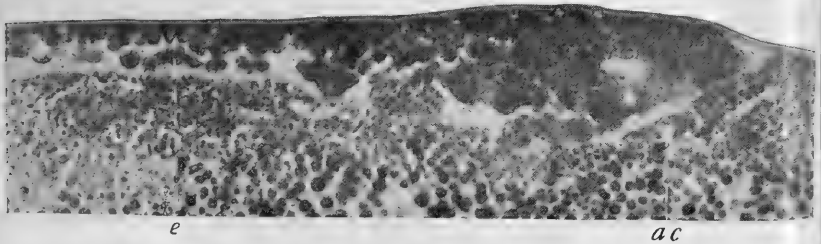

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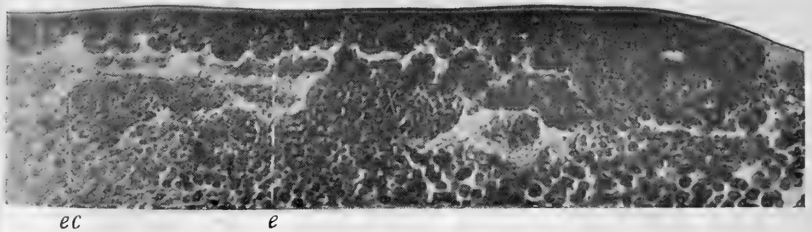

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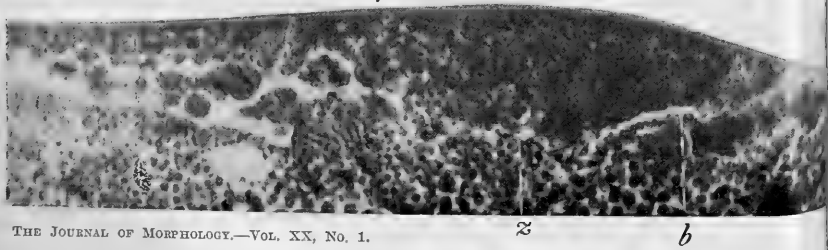

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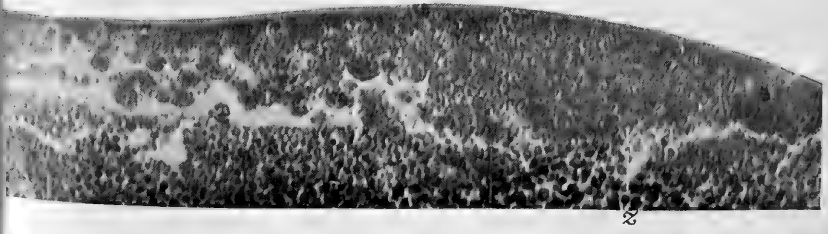


Plate VI.

Fig. 42. A portion of the anterior half of a median longitudinal section from the blastoderm represented in Fig. XV. At "*e*" is the anterior limit of the entoderm. ×120.

Fig. 43. A portion of the posterior half of the preceding section. × 120.

Fig. 44. The enlarged central portion of a section from the same blastoderm as Figs. 42 and 43. Note especially the epithelial character of the ectoderm, the grouping of the entoderm cells, and the granular contents of the cavity. × 184.

Fig. 45. From a median longitudinal section of an unincubated egg. It shows the anterior limit of the entoderm in its forward growth. The remains of the subgerminal cavity (*sg*) is entirely free from yolk mass. × 245.

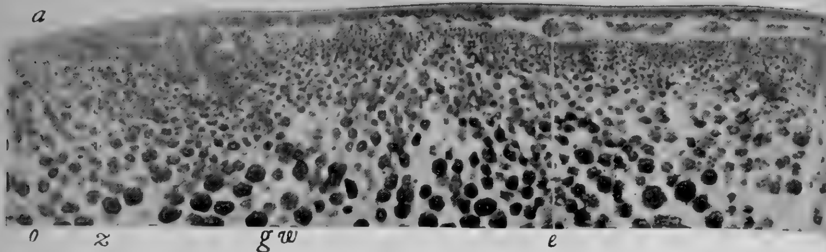
Fig. 46. The central part of a longitudinal section from a blastoderm taken three hours after incubation. It shows the fragmentation of the yolk lying beneath the floor of the cavity. These yolk masses (*m*) are non-nucleated. × 246.

Fig. 47. The anterior portion of a longitudinal section from a blastoderm taken forty hours after fertilization. The remains of the subgerminal cavity not yet penetrated by the entoderm (*e*) is full of yolk masses, some of which are undergoing fragmentation (*dm*). × 245.

Fig. 48. The right side of a median transverse section taken one hour after incubation. The lateral edge of the entoderm is shown at "*e*" and the inner margin of the germ-wall at "*y*." The space between these two points can be followed along the entire right side, showing that the fusion between the lateral margin of the entoderm and the inner edge of the germ-wall had not yet taken place. × 245.

Fig. 49. The central part of a section taken through v-v′ of Fig. VIII. At "*s*" is shown a segmentation cell that has loosened and sunk down from the underside of the ectoderm. × 245.

42



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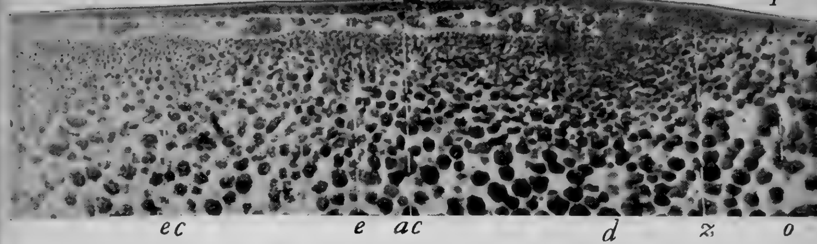

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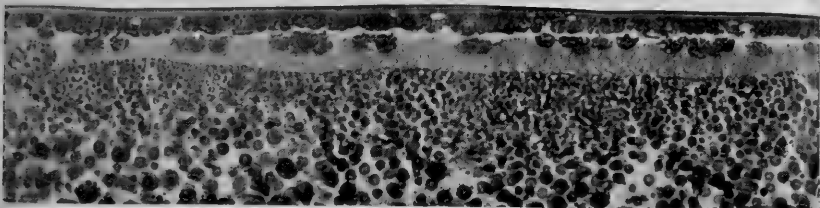

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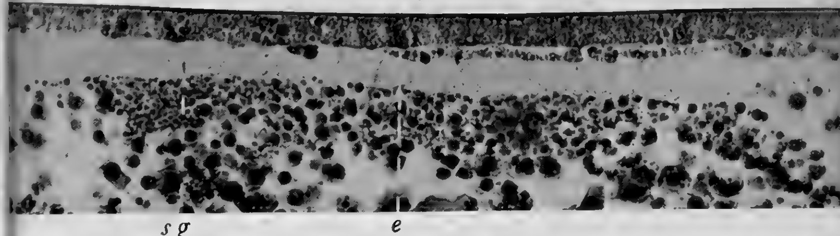

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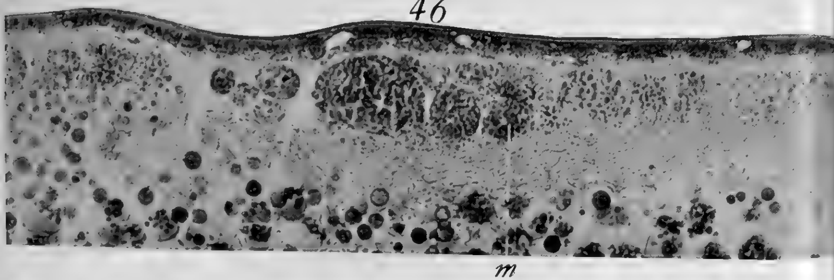

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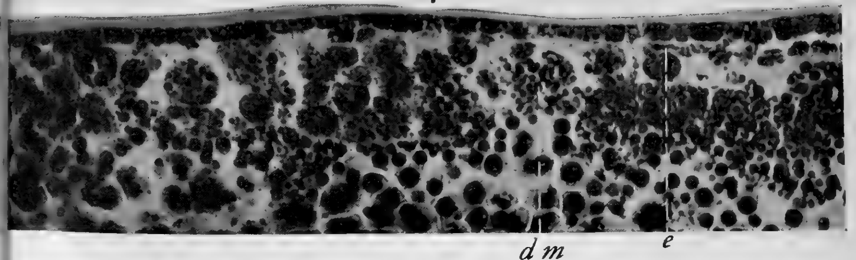

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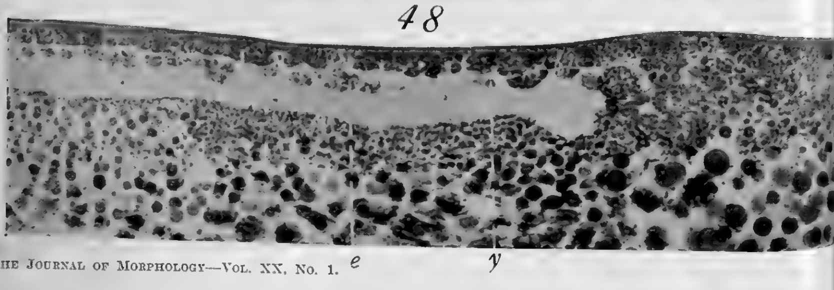

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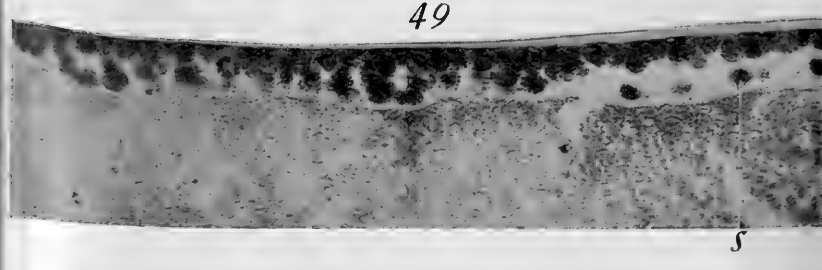

Plate VII.

Fig. 50. This embryo shows the result of the operation described in Experiment II (page 93). The injury was made on the posterior edge of the dorsal lip, thirty-five and three-fourths hours after fertilization. The egg was then incubated for forty-nine hours. The anterior end of the embryo is normal in every way, and nineteen pairs of somites are developed. The depth to which it was necessary to focus the microscope in order to obtain an image of the injured material in the entoderm can be seen by the fact that the somites are out of focus. $\times$ 30.

Fig. 51. A transverse section through the injured region of the preceding embryo (Fig. 50, op). The affected cells lie in the entoderm. $\times$ 95

Figs. 52, 53, and 55 are all from an unincubated blastoderm. Fig. 52 shows the entoderm and part of the ectoderm above, and a "free nucleus" at *n* lying on the floor of the cavity, which contains many small granules. $\times$ 688.

Fig. 53. It shows two small nucleated cells (at *s* and *s*), which are doubtless wandering entoderm cells. There are also many large yolk masses in the cavity. $\times$ 500.

Fig. 54. This shows the result of the operation in Experiment VI. The injury was made thirty-five hours after fertilization, and the egg then incubated for forty-eight hours. There are twelve pair of mesoblastic somites present, the development being slightly retarded. $\times$ 20.

Fig. 55. This shows a large multinucleated yolk mass, in which most of the nuclei are degenerating. $\times$ 500.

Fig. 56. This shows the result of the operation in Experiment X. The injury was made on an unincubated blastoderm, and the egg was then incubated for twenty-three hours. The embryo is normal in every way. $\times$ 25.

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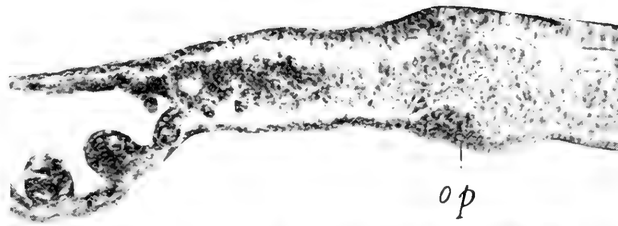

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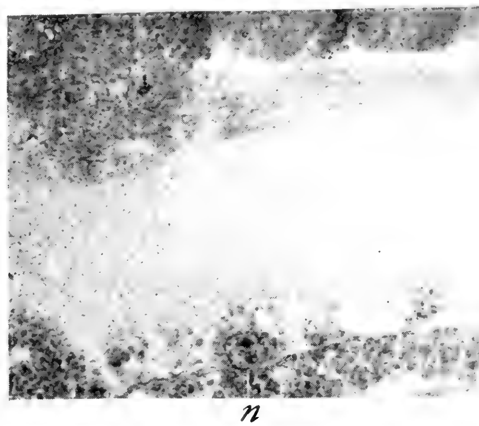

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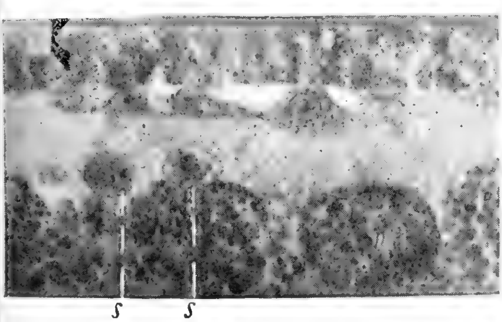

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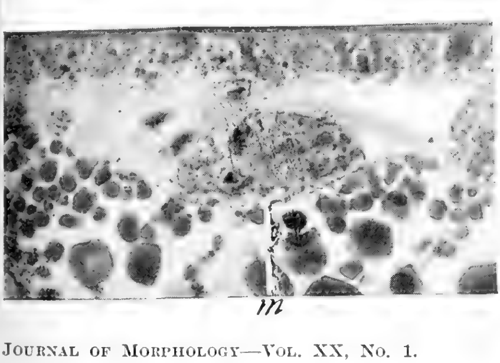

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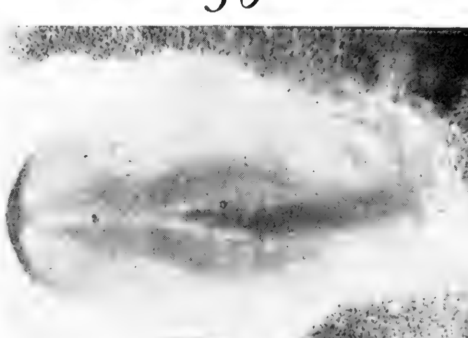

Plate VIII.

Fig. 57. Transverse section through the injured head-fold of the embryo shown in Fig. 59. The main group of injured cells is at *op.* $\times$ 95.

Fig. 58. Transverse section through the injured region of the embryo shown in Fig. 60. The needle has destroyed a considerable portion of the primitive streak material, and has also disturbed the underlying entoderm. The folding of the lateral portions of the entoderm is an artifact. $\times$ 95.

Fig. 59. This embryo was operated on thirty-four and one-third hours after fertilization, and then incubated for thirty-four hours. The injury was made in the center of the dorsal lip at a distance from the posterior margin equal to about half the width of the needle (see Fig. XVI, *a*). Posterior to the head fold the embryo is normal in every way. $\times$ 21.

Fig. 60. The operation was performed on a freshly laid egg, which was then incubated for twenty-six and three-fourth hours. The injury was made on the boundary between the areas opaca and pellucida, in line with the axis of the future embryo (see Fix. XVI, *b*). There are twenty-five sections posterior to the injury that show a characteristic primitive streak structure. $\times$ 21.

Fig. 61. Transverse section through the injured region of the embryo shown in Fig. 68, Pl. X. Just one-half of the embryo has been affected by injury. $\times$ 95.

J. Thos. Patterson.

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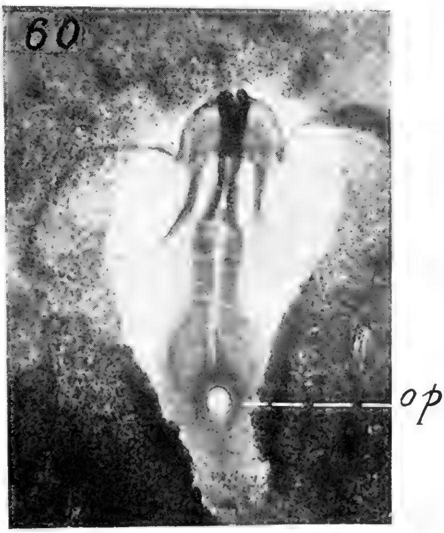

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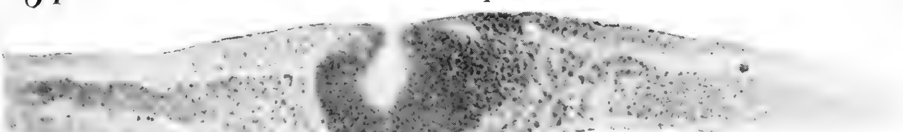


Plate IX.

Fig. 62. Transverse section through the injured region (mid-brain) of the embryo shown in Fig. 63. Only the right neural fold is affected by the operation. × 87.5.

Fig. 63. This embryo shows the result of an operation made ten degrees to the right of the median line, in the margin of the dorsal lip. The injury was made thirty-four and three-fourth hours after fertilization, and then incubated for thirty-six and three-fourth hours. × 20.

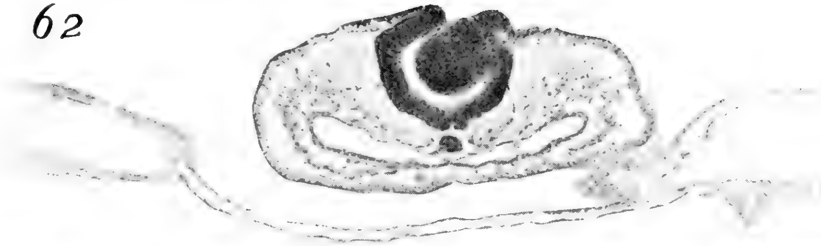
Fig. 64. This shows the result of the operation for Experiment VII. The injury was made thirty-six and three-fourth hours after fertilization, and the egg was then incubated for thirty-six hours. The operation was made just after the closing of the blastopore (see Fig. V, *K*). × 21.

Fig. 65. This shows the left side of a transverse section of an unincubated hen's blastoderm. The figure is introduced to show the rounded condition of the region of overgrowth, which is raised up from the yolk. I am indebted to Professor George Lefevre for his generosity in sending me the series from which this photograph was made. × 128.

Fig. 66. Posterior end of a median section from the blastoderm described in connection with Experiment I (see text for description). × 120.

Fig. 67. A portion of the same section taken just anterior to the preceding. × 120.

62



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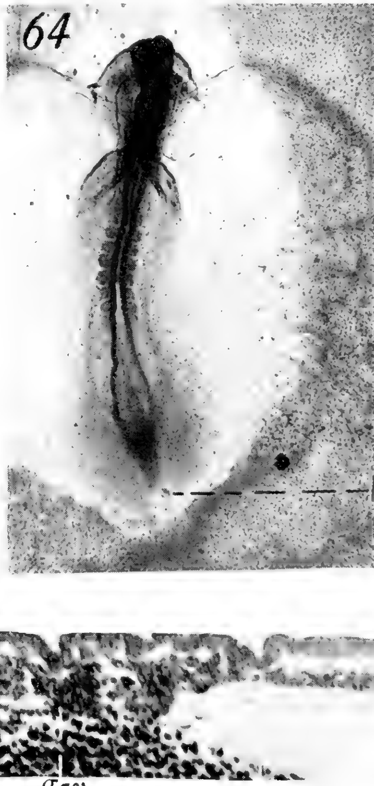

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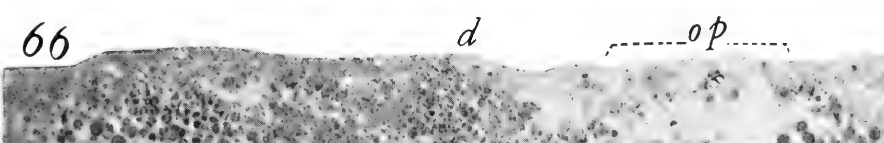

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Plate X.

Fig. 68. This embryo shows the result of an injury made on an unincubated blastoderm, at about twenty degrees to the right of the median line on the boundary between the areas opaca and pellucida. The egg was incubated for forty hours. The arrow shows the path traversed by the mass of injured cells, as indicated by the small groups of dead cells. The posterior end of the embryo is bent to the right. The curvature is due doubtless to unequal growth of the cells on the two sides. (For a transverse section through the injured region of this embryo, see Fig. 61). $\times$ 20.

Fig. 69. The operation was of the same nature as the preceding, except that it was made about thirty degrees to the right of the axial line instead of twenty. The egg was incubated for twenty-four and one-half hours, and the injury is situated slightly more posteriorly than in the preceding experiment. $\times$ 25.

Fig. 70. In this embryo the injury was made between three and four hours after incubation had begun, at about forty-five degrees to the right of the axial line on the boundary between the areas opaca and pellucida. The egg was then incubated for thirty-six hours. The embryo is normal in every way and the injured spot is in the vascular area, about half way between the *sinus terminalis* and the pellucid area. $\times$ 18.

Fig. 71. The injury in this blastoderm was made on the posterior margin forty-five degrees to the right of the median line. The operation was performed thirty-three and one-half hours after fertilization, and the egg was then incubated for thirty-six hours. The group of injured cells has been brought into the axis of the embryo. $\times$ 21.


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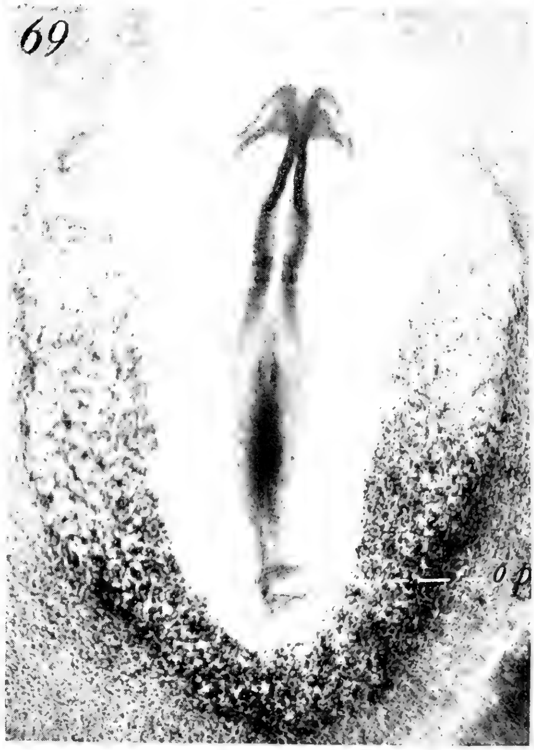
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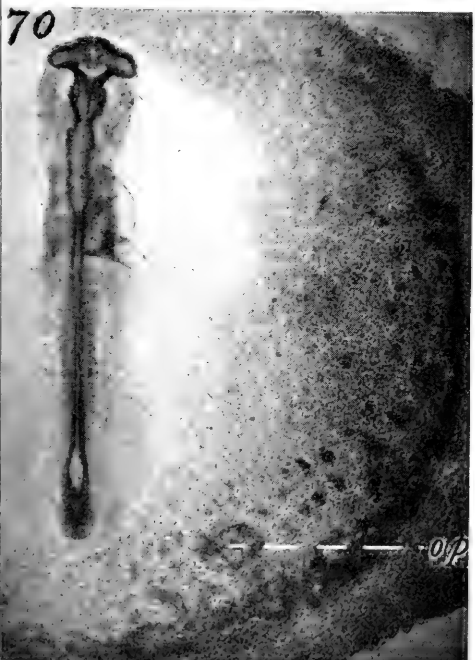
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NUTRITION OF THE OVUM OF SCOLIA DUBIA.

BY

WILLIAM A. KEPNER.

WITH 2 PLATES.

In September, 1906, many larvæ of Allorhina nitida were working in the sandy soil and sparse sod of a certain part of the University campus. These larvæ were being attacked by the very few female Scolia dubia attended by the more numerous males. Specimens of both the larvæ and the wasp were then collected and later sent to Dr. L. O. Howard, Chief of Bureau of Entomology, Washington, D. C., where they were identified by Dr. F. H. Chittenden as "Scolia dubia and its host the 'grub-worm' or white grub, Allorhina nitida, commonly known as the 'green June beetle' or 'fig eater.' "

In September, 1907, the larvæ of Allorhina nitida were very scarce, but in their place an extensive swarm of Scolia dubia appeared. Of these the females, as they crept over the soil or burrowed into it, were caught and various tissues fixed for future study. The material for this work was all taken from adults. The exoskeleton of the adult is so thick as to make it impossible to section the ovaries in place. The ovaries were, therefore, removed from the body cavity with care and fixed. Certain ovaries were fixed in aceto-sublimate two hours, others in chrom-aceto-formalin one hour, and a third supply in Flemming's stronger fluid five hours. All sectioning was done in paraffin. The sections were made 5, 10 and 15 microns thick and mounted in series. All staining was done on the slide. Borax carmine, iron hæmatoxylin, safranin, thionin and methylen green were used in staining. Tissues fixed in chrom-aceto-formalin or in Flemming's stronger fluid and stained with iron hæmatoxylin gave the best results.

The ovary of Scolia dubia is a paired structure. Each member of the pair consists of four ovary tubules which lead into a common oviduct. The histological structure of these tubules is characteristic

of the ovary tubules of many insects. Each tubule is anchored with a terminal filament to the peritoneal wall. This filament abuts against a region filled with certain primordial cells. This region of the tubule is called the terminal chamber (Fig. 1). Leading from the terminal chamber is a chain of follicles composed of nurse-cell follicles alternating with egg follicles. In the adult this chain of follicles extends from terminal chamber to the common oviduct.

The terminal filament is composed of spindle-shaped cells which tend to lie parallel to the axis of the filament. Their outline is not well defined. The cytoplasm is densely granular, which makes them conspicuously different from the cells of the terminal chamber (Figs. 1, t. f., and 7, t. f.). The nuclei are oval and have an evident reticular structure; they measure 6 to 8 microns long.

While it is the writer's opinion that the terminal filament is but a peritoneal process serving as an anchorage to the ovary tubule, and having nothing to do with either the origin or the nutrition of the oöcytes, certain early writers have held it to be a more important structure and that by a repeated division of its cellular elements it gives rise to groups of cells which form the primitive elements from which the cells of follicle epithelium, nurse cells and ova were differentiated. The results of more recent investigators point decidedly away from this view. Köhler, '07, deals with this particular feature of the insect ovary and claims that "Bei den Hemipteren ist der Endfaden meist von der Endkammer durch die Tunica propria getrennt. Auch dort, wo dies nicht dar Fall ist, wo sich der Endfaden als Fortsetzung der die Endkammer ausscheidenden Epithelzellen zeigt, besteht eine sharfe Abgrenzung der Epithelzellen des Endfadens gegen die Geschlechtszellen der Endkammer. Der dient ausschliesslich als Aufhängeband."

In Scolia dubia the tunica propria does not completely separate the terminal filament from the cells of the terminal chamber. There is, however, a rather distinct differentiation between the two regions. The cells of the terminal filament stain more deeply than those of the terminal chamber. Between the two regions there can be found no intermediate zone marked by mitotic divisions or other transitional features. (Fig. 7.)

A marked feature of the region in the vicinity of the terminal filament is that peculiar structures appear within the terminal chamber. There are here to be seen oval cells, deeply staining, measuring 10 by 15 microns. Their nuclei are oval and their cytoplasm dense and finely granular. Except for rounded bodies within it the cytoplasm appears homogeneous (Fig. 7 a). At b, Fig. 7, is seen an irregular space containing what may be the remains of one of these peculiar cells broken down. At places smaller cells with what appear to be two, three or more nuclei within each may be found. These figures suggest quite strongly the series shown in Figs. 3 to 19, Taf. I of Will, '86, which this author has interpreted as phases in the development of nurse cells, ova and follicle cells from "oöblasts." The phenomena pictured by Will were at once taken up in dispute by Korschelt, '87. Stuhlmann, Blochmann and Schneider also denounced Will's theory. Among the later writers to dispute Will's interpretation was De Bruyne, '98, who records that some of the cells of the "germigène" undergo a histological transformation characterized by the appearance in the protoplasm of spheres of compact structure serving to support chromophilous fragments more or less numerous. These transformed cells lose their boundaries and their products of degeneration scatter in the cavity of the terminal chamber between the cells which have preserved their general aspect. "Ces produits serrés entre les cellules restées intactes, penetrent ou arrivent par englobement jusque dans celles que l'on reconnaît déjà comme étant les futurs éléments ovulaires et vont y contributer à leur accroissement: il s'agit d'une degenerescence spontanée, debutant et s'achevant sans l'intervention de cellules sanguines et d'une disparition subsequent par englobenment de la part de la cellule-œuf, qui joue ainsi le rôle de phagocyte. Les boules du protoplasme nées dans les cellules nutritives correspondent donc aux produits des oöblasts (Will) et leur partie chromatique est le noyau né, d'après cet auteur, par bourgeonnement de ces mêmes oöblasts. Non seulement des cellules nutritives peuvent, en dégenérant, donner lieu à des substances d'accroissement pour l'ovule, mais l'epithelium aussi peut secreter de ces boules."

Giardina, '01, shows convincingly that the origin of the nurse cells

and oöcytes are quite unlike what Will believed it to be. Giardina describes and figures clearly that an oöcyte and its attending group of nurse cells arise by a series of differential mitoses from a primordial reproductive element of the terminal chamber.

The peculiar features shown in Fig. 7 are found only at the distal end of the terminal chamber. They suggest different stages in the degeneration of certain cells (Fig. 7, a and b). The oöcytes in this region tend to be vacuolated about the periphery and are unusually large. (Compare Figs. 2 and 7.)

The specimens of Scolia dubia were taken very late in their breeding season. It is not probable that all the contents of the terminal chamber would be demanded by the rapidly closing season. This would lead us to expect degeneration phenomena within the distal end of the terminal chamber.

Fig. 7 shows an apparent relation of position existing between one of the unusually large oöcytes and a mass of degeneration products. This is but accidental; for many such bodies are found lying remote from any oöcyte. These products in Scolia dubia, therefore, are considered to have nothing to do with the nutrition of the oöcyte as De Bruyne in the above quotation suggests, but to be mere degeneration products concomitant with the close of the season.

Besides the degeneration cells the terminal chamber contains the primordial elements of future follicle epithelium, young nurse cells and young ova or oöcytes.

Throughout the history of the ovum within the ovary tubule its size increases greatly. The smallest and youngest egg cells within the terminal chamber are about 25 microns long and 20 microns thick. They rapidly grow during their passage down the tubule until they become 1,000 microns long by 350 microns wide. They are throughout this growth highly plastic and readily conform to any irregularities of surface.

During this remarkable growth of the cell body the nucleus remains, so far as comparative measurements of different nuclei show, constant in size. The nuclear contents are highly achromatic except for an irregular mass of chromatin which is always eccentrically situated. This mass is least assembled in the youngest nuclei.

It is interesting to note that the nuclear pattern shows little or no change throughout the entire nutrition of the egg cell.

The terminal chamber of Scolia dubia is limited by a well defined tunica propria, which is composed of a layer of flattened cells similar to the cells of the terminal filament except that they stain less. A similar tunica propria extends throughout the extent of the ovary tubule.

Beneath this layer within the terminal chamber are found the irregularly disposed primordial follicle cells. These cells are crowded within the interstices between the other elements of the terminal chamber. They are the smallest elements within the terminal chamber, their oval nuclei measure about 7 to 9 microns in length. These cells are more or less polygonal in outline and not clearly defined. They are rapidly proliferated by mitoses and as they thus become crowded toward the proximal end of the chamber they assume a spindle shape and lie at right angles to the length of the terminal chamber (Fig. 1, f. c.).

Here they are assembled about an ovum or its attending group of nurse cells to form the follicle epithelium of an egg follicle or the epithelium and scaffolding of a nurse follicle. When entering the formation of the scaffolding of a nurse follicle they have irregular shapes; but in all follicle epithelia the cells are columnar and stand at right angles to the surface of the ovum or nurse cell mass.

Distal to each nurse follicle, follicle cells assemble to separate the newly formed nurse follicle from the egg follicle about to form. In this manner the follicle epithelium develops as a continuous epithelium from the terminal chamber to the end of the ovary tubule; at this latter region it abruptly becomes a much taller columnar epithelium that is more or less convoluted to form the thickened wall of the proximal end of the tubule just as it passes into the structure of the common oviduct. To this thickened region the French give the name "calycule" and the Germans "Wandverdickung."

In the growing follicle cell proliferation continues by mitosis. The nuclei of such follicle cells are oval to spherical and do not

stain deeply. About the follicles they elaborate a homogeneous substance called the chorion. Within the folds between the follicles the chorion forms a partition (Fig. 5). As the egg follicle nears its ultimate size, the entire epithelium elaborates a clearly defined cuticle. The cells now become much shorter. The chromatin of each nucleus becomes assembled into four or six irregular masses which stain very deeply. The process continues until the epithelium is reduced to a very thin cytoplasmic layer with greatly flattened deeply staining nuclei.

In Scolia dubia the follicle epithelium is concerned chiefly in the production of the chorion and does not secrete food products for the egg cell; though in the ultimate breaking down of the nurse follicle their disintegration products are most probably taken into the egg cytoplasm for food. Its chief function, therefore, appears to be the formation of the chorion.

The follicle epithelium is, however, in a secondary manner concerned with the nutrition of the egg cell. Bambeke, '97, found that the egg nucleus of Pholcus, at the time yolk is being elaborated out of the material entering the egg cytoplasm from the nurse follicle becomes irregular in contour and at the side nearest the deposit of yolk gives out many slender pseudopods, as if to increase the nuclear surface that many take part in the elaboration of deutoplasm. De Bruyne, '98, quotes Korschelt as saying that the products secreted by the nurse cells are carried to the germinal vesicle and completely transformed by it. Rabes, '00, in speaking of the egg of Rhizetragus solstitialis L., says: "Jedenfalls unterliegt es keinem Zweifel, dass in der Eizelle besonders in der Zeit ihres Wachstums, eine ungemein innige Wechselbeziehung zwischen Kern und Zellplasma besteht, die sich am auffallendsten in den Form- und Lageveränderungen des ersteren zu erkennen giebt."

As over against the apparent remarkable nuclear activity observed in the forms studied by the above investigators, the egg nucleus of Scolia dubia shows no change of contour; its chromatin pattern is comparatively constant throughout the complete nutrition series, and except for its position there is indicated no relation between the egg nucleus and the handling of the food substances from the nurse cells.

In this connection it is interesting to note that there are present at the distal pole of the egg cell a number of smaller nuclei than the egg nucleus, which have a conspicuous nuclear net-work and nucleolus. Their position is a strong indication that they have a rôle to play in the elaboration of deutoplasm or yolk as the secretion of the nurse cells is passed into the egg cell.

Similar nuclei are described by Gross, '01, for the ovum of Vespa vulgaris. The following is quoted from this paper: "In alten Eiern findet sich ausser dem Keimbläschen constant eine Anzahl kleiner Kerne. Dieselben sind zuerst von Blochmann (1886) bei Ameisen und Wespen beobachtet worden. Sie liegen Anfangs in der Nähe des Keimbläschens, entfernen sich aber bald von ihm, rücken an die Peripherie des Eies und bilden hier eine Lage um den grössern Theil des Dotters (Fig. 186). Blochmann nahm an, dass diese Kerne von Keimbläschen abstammen. Korschelt (1886) der ähnliche Gebilde von Musca beobachtete, lässt die Frage nach ihrer Herkunft offen. Bei Hymenopteren, die ich untersuchen konnte, ist die Abstammung der genannten Kerne eine andere und weniger auffallende als die von Blochmann augenommene." * * "Beginnen nun die Nährzellen ihren Inhalt in das Ei zu entleeren, so gelangen auch die Epithelkerne in den Dotter und bleiben hier, nachdem sie die eben erwähnten Ortsveränderungen durchgemacht haben, noch lange erkennbar. Korschelt (1886) meint, dass diese Vorgänge mit der Dotterbildung zusammenhängen. Auch mir scheint dies sehr warscheinlich zu sein. Die Keimbläschen der Hymenopteren sind auffallend klein. Da nun aber, wie Korschelt (1891) gezeigt hat, dem Eikern der Insecten eine wichtige Rolle bei der Umwandlung des dem Ei zuströmenden Nährmaterials im Dotter zugeschrieben werden muss, so könnten die ins Ei gelangten Epithelkerne dem Keimbläschen zu Hülfe kommen und sich mit ihm in die erwähnte Function theilen."

By a careful study including many measurements of egg nuclei no evidence was obtained in support of the above view of Blochmann that these nuclei were given off by the egg nucleus. On the other hand several cases have been found, in which certain follicle nuclei had assumed the appearance of these nuclei and appeared to have

been just about to enter the egg cytoplasm. It appears quite probable, therefore, that these migrated nuclei, or as Gross, '01, calls them, "einwandernde Epithelkerne," have been furnished by the follicle epithelium.

The migration suggests the observations made by Metcalf on the ova of Salpa; but the two phenomena differ functionally. In Salpa the nuclei of the follicle epithelium are taken into the egg cytoplasm primarily as a supply of food for the ovum. In Scolia dubia, on the other hand, they have migrated primarily to become the handlers of food material and only when this primary function is terminated do they function in the same manner as the migrated nuclei of Salpa.

At the beginning of the second phase of nutrition when the nurse cells are about to send food material into the egg cell, but one or two of these migrated nuclei are present (Fig. 6). As the egg cell grows and the elaboration of yolk is begun, these greatly increase in number. Along the distal periphery of the egg cell they then form a closely packed layer of clearly defined, rounded to oval nuclei, each of which has a definite nuclear reticulum and a conspicuous nucleolus. They lie in less numbers about the entire periphery of the egg cytoplasm. Those found below the distal fourth of the egg cell are smaller and stain more readily. Have all of these many nuclei come from the follicle epithelium?

In Scolia dubia during the early stages and in favorable places within older egg cells, groups of ova are met with in which one or two large migrated nuclei are found surrounded by smaller ones (Fig. 8). In many other cases large nuclei are found, on which there is a partial constriction which divides the nucleus into a large and a small lobe. In all such cases *two* nucleoli are present; while only few nuclei with two nucleoli and with no constriction were observed (Fig. 9.) These observations have led to the interpretation that the migrated follicle nuclei propagate within the egg cytoplasm by means of amitosis, which results in each case in daughter nuclei of unequal size. In any cases where the egg nucleus is unaided by other nuclei Dotterkerne or yolk nuclei are found. These are so frequent that to give examples in this connection is uncalled for. In the ovum of Vespa vulgaris described by Gross, '01, as

having the migrated nuclei, no yolk nucleus is described. Similar conditions are met with the ova of ants and wasps described by Blochmann, '86, and in the ova of Musca described by Korschelt, '86. That ova with these migrated nuclei to aid them in the handling of food material entering them from the nurse cells have in no case a yolk nucleus is significant. It indirectly suggests that the yolk nuclei of other ova are but accumulations of food material that has entered the ovum more rapidly than the cytoplasm with a single nucleus was able to transform it into yolk.

These nuclei are the preparers of the food and are concerned in but a secondary manner with the nutrition of the ovum in Scolia dubia.

We believe that we are warranted in recognizing two phases in the nutrition of the ova of this wasp. The first phase runs its course within the terminal chamber. The second involves the complete history of the nurse follicle after it leaves the terminal chamber. The second phase, therefore, lies entirely outside the terminal chamber.

The follicle cells and their derivatives are now considered somatic cells. Köhler, '07, writes: "Die Zusammengehörigkeit der einzelnen Zellen regelt sich folgendermassen: Als gemeinsamen Ursprungs sind anzusehen: die Zellen des Peritonealepitheles, des Endfadens, des Eiröhrenstieles, des Endkammer- und Follikelepithels. Diesen somatischen Zellen stehen gegenüber die Geschlechtszellen, d. h., die Nährzellen und Keimzellen."

Nurse cells and egg cells have long been known to be the chief cells of insect ovaries. Stein, '47, first recognized them as two distinct elements; but considered them to be masses of homogeneous protoplasm. Meyer, '49, recognized the cellular nature of nurse cells and egg cells.

Folsom, '06, uses a diagram from Lang's Lehrbuch to illustrate his description of three types of ovaries of insects. The first type is represented by ovary tubules composed of a chain of egg follicles without nurse cells. The second type includes the tubules composed of alternating egg and nurse follicles which arise out of a terminal chamber. To the third type belong such ovary tubules as are com-

posed of a series of egg follicles remaining in connection with certain nurse cells, that do not leave the terminal chamber, by means of a protoplasmic strand, which the Germans have called Dotterstränge or Dottergänge and which Lubbock has named yolk ducts. The third type involves, therefore, only the nurse cells as they lie within the terminal chamber. Later it will be shown that Scolia dubia in its nutrition combines the second and third types.

Leuckart, '53, and Lubbock, '60, were the earliest writers to describe the nutrition of the ovum by means of yolk ducts connecting the ovum with the nurse cells of the terminal chamber. Claus,C., '64, describes for Apis platanoides a very characteristic "Dotterstrang" leading from groups of three to six nurse cells to the ovum. Gross, '01, describes for Asopus bidens yolk ducts between the follicle ova and nurse cells within the terminal chamber. Wielowieyski, '06, in describing the ovary of the hemipter Pyrrhocoris apteris says: "Die Dotterzellen sind in einer Endkammer vereinigt und werden mittelst feiner, im Karkraume derselben verlaufender plasmatischer Ausläufer mit ebensolchen plasmatischen Ausläufern der Eizellen verbunden, so dass ein Ernährungssystem entsteht, in welchem dei einzelnen Dotterzellen mit den Eizellen direkt kommuniziren." Kohler, '07, in the ovary of Nepa cinerea describes large yolk ducts which lead from the terminal chamber and give off lateral branches to individual ova.

All the above forms show a nutrition involving nothing but yolk ducts and occurring only through the yolk duct. In Scolia dubia there are two phases of nutrition. The first phase is accomplished within the terminal chamber through short yolk ducts of a peculiar type. Not all the nurse cells take part in this first nutrition phase. The second phase takes place from the nurse follicles and involves all the nurse cells.

The presence of extensive yolk ducts between nurse cells and ova have in the past been taken as sufficient evidence that this was a feature pertaining to the nutrition of the ova. The inference that a nutrition phase of the ovum of Scolia dubia ensues within the terminal chamber is based upon (*a*) the presence of short yolk ducts, and (*b*) the condition of the nuclei of such nurse cells as are attached by these ducts to the ova.

Throughout the extent of the terminal chamber ova are distributed. Except for the one or two at the distal end of the terminal chamber, which are surrounded by apparent degeneration products, all the ova show extremely short yolk ducts which connect them with certain of their attending nurse cells. These yolk ducts have a wall, which appears as a ring formed by the blending or coalescing of a region of the cell membranes of nurse and egg cells, and a central core of cytoplasm. The wall when seen in profile is extremely short, measuring in the youngest stages .25 micron and at its maximum size 1 micron (Figs. 2 to 5). When seen in transverse section it appears as a ring with a comparatively wide body and a diameter of from 2.5 to 4 microns. The wall of the duct stains intensely with all the stains employed as mentioned above. In all the preparations it reveals a homogeneous structure and shows no constituent granules such as Giardina, '01, describes. The cytoplasmic core is not conspicuous except in the late phases of its duration when it becomes greatly elongated (Fig. 5). Unlike the similar structure described by Giardina for Dytiscus this core showed no affinity for particular stains which would give a differential stain.

As the ovum develops and passes proximally through the terminal chamber, the ring-like yolk ducts become more prominent and their core of connecting cytoplasm may be seen. The maximum development is reached before the nurse follicle is formed.

The yolk ducts appear first on all sides of the ovum. After their maximum development is attained, the proximal ones separate from the ovum, and the nurse cells thus freed take a position distal to the ovum which they attend, and enter the cell group of the developing nurse follicle. Thus with the near approach of the complete formation of the nurse follicle, only a few of the nurse cells—the distal ones—retain their yolk ducts (Fig. 5). These few ducts are eventually severed by the crowding of all the nurse cells away from the ovum into the completed nurse follicle (Fig. 6).

The nurse cells may be considered gland cells which secrete material for the ovum. The nuclear pattern of the cells having yolk ducts differs strikingly from that of the other nurse cells. These chromatin differences furnish additional evidence that the yolk ducts above described have to do with a nutrition phase.

Korschelt, '91, indicated a difference between the nuclei of gland cells that were actually secreting and those that were at rest. He says: "Nach seiner Darstellung enthalten die Kerne secretgefüllter Drüsenzellen ziemlich grobe Chromatinkörner, welche durch Fäden unter einander verbunden sind. Es ist ein derbes Chromatinnetz vorhanden, wie Hermann es bezeichnet. Mit der Entleerung des Secrets findet eine Aenderung der Structurverhältnisse des Kernes insofern statt, als die derben Chromatinbrocken aufgelöst werden und an ihrer Stelle ein feines zierliches Chromatinnetz tritt, das je nach dem Stadium der Secretausstossung noch eine geringe Menge verkleinerter Chromatinbrocken beherberge, bis dieselben in der volkommen secretleeren Zelle gänzlich verschwunden sind. Diese Beobachtungen lassen auf die anschaulichste Weise eine Beziehung der Kerne zu der Thätigkeit der Zelle erkennen." Woltereck, '98, De Bruyne, '99, Rabes, '00, Gross, '01, and others have followed Korschelt in this interpretation. Gross says: "Korschelt (1891) hat entschieden Recht, wenn er diese Erscheinungen als Anzeichen einer starken Betheiligung des Kernes an der secretionschen Thätigkeit der Zelle betrachtet."

In this connection, therefore, it is of interest to note that the nuclei of those nurse cells not attached to the ovum by means of yolk ducts have their chromatin concentrated (Figs. 3, 4, 5). The nurse cells in the distal region of the terminal chamber which have yolk ducts have their chromatin distributed upon a more or less definite, open, reticular net-work (Fig. 2). As the ovum passes down the terminal chamber carrying with it the nurse cells the chromatin in the attached nurse cells becomes finely granular and evenly distributed throughout the nuclear cavity (Figs. 3, 4). In this way the chromatin of these nurse cells behaves in a manner characteristic of many secreting gland cells.

During this phase of nutrition vacuoles appear within the cytoplasm of the ovum together with deeply staining granules. These are held to be nutrition products.

The first phase of nutrition ends with the breaking of the last yolk ducts and the formation of the complete egg and nurse follicles.

The nurse cells of the completed follicles, except for an occasional

follicle cell, lie against each other in a compact mass. At the proximal base of the follicle is found a core of follicle epithelial cells. Into this cellular core the ovum sends a cytoplasmic process. The nurse cells have in the meantime undergone an intermediate period of rest and growth (Fig. 6).

With the approach of the second phase of functional or secreting activity the migrated follicle nuclei (described at p. 132) appear within the cytoplasm of the ovum (Fig. 6). The chromatin of the nuclei of the nurse cells becomes evenly distributed. This chromatin feature remains little changed in those cells that have functioned through yolk ducts. In the others the charge of chromatin distribution travels as a wave from the proximal region distally throughout the follicle. The distal nuclei, therefore, are the last to show this chromatin feature.

The nurse cells continue to grow. Their nuclei become more or less irregular in contour. Vacuoles appear in their cytoplasm. Within the ovum the migrated nuclei become abundant and lie at the pole next to the nurse follicle. A large irregular vacuole appears within the egg cytoplasm as the evident recipient of the secretion of the nurse cells (Fig. 10). With these appearances the second phase of nutrition may be considered well under way.

The secretion continues at the expense of the nurse cells. The cytoplasm of some of the proximal cells breaks down. The remaining cells become loosely disposed within the follicle. As the process continues, a wave of cytoplasmic disintegration passes more or less regularly distally through the nurse follicle which is closely followed by the disintegration of the nuclei. In the meantime the nurse follicle collapses (Fig. 10).

The follicle epithelium cells between ovum and nurse cells become loosely arranged so that the secretions of the nurse cells pass freely into the greatly enlarged ovum. These secretions continue to form a vacuole in the multinuclear cytoplasm at the distal pole of the egg cell. By the interaction of the contained nuclei and cytoplasm the material thus taken up is transformed into yolk spherules which are deposited in the proximal half at the periphery of the ovum (Fig. 10, *y. g.*). The deposition of yolk continues until all the cytoplasm

has become a reticular meshwork supporting many yolk spherules and the egg nucleus.

The migrated nuclei finally disintegrate to form part of the food supply of the ovum. About the ovum the follicle epithelium builds a complete chorion. These phenomena mark the end of the second phase of nutrition.

The final disintegration of the nurse cells takes place wholly within the nurse follicle. De Bruyne, '98, observed in Dytiscus that after the cytoplasm of the nurse cells had disintegrated the nuclei of these same cells were taken *in toto* into the cytoplasm of the ovum to be consumed by it. Paulcke, '01, discovered a like fate of the nurse cell nuclei in the ovaries of Apis mellifica. In these cases the disintegration of the nurse cell nuclei is similar to that of the migrated follicle nuclei of Scolia dubia.

With this the second phase of nutrition is completed and the ovum with its cytoplasm completely charged with yolk is delivered into the oviduct.

The insect ovary has been a subject of so much scientific investigation during recent years, that except for his observations confirming Giardina's interesting observation, the writer would not be justified in adding to the extensive bibliography. Giardina, '01, was the first to clearly define two phases of nutrition in the ovum of an insect. His observations on Dytiscus has hitherto stood without confirmation. Concerning his priority in this observation he says: "E giustizia notare che, gia nel 1880, Tichomiroff descrisse nel-Bombyx mori un' aperture centrale di communicazione nella parete divisoria tra l'uova e la camera nutrice, e che da questa apertura vedeva penetrare nell' uova sostanza granulosa, simile alla sostanza delle cellule vitellogene. Quantunque la descrizione non sia perfettamente corrispondente alla realta, pure non vi puo esserdubbio che essa si riferisca alle communicazioni protoplasmatiche ova descritte. Anche il Korschelt (1889) non dubitava che delle communicazione tra l'oöcite e le cellule nutrici dovessero esistere, ma gli argomenti da lui adotti non erano molto convincenti."

"Simili connessione attivamente alla nutrizione dell' uovo, e rendono poco verosimile l' opinione del De Bruyne (1898), che esse vi

partecipino solo passivamente, lasciandosi divorare dell' oöcyte per via di fagocitosa."

Fundamentally the writer's observations on the ovary of Scolia dubia during the first phase of nutrition are in accord with the findings of Giardina in the ovary of Dytiscus. The heavy walls of the yolk ducts which Giardina describes as being composed of a series of granules were homogeneous structures in Scolia dubia. Within the cytoplasmic core there were no traces of fibrillation, nor could any differential stain be made of this part of the cytoplasm. In these structural details Giardina's Dytiscus material differs from the specimens of Scolia dubia.

Giardina is content to establish the presence of a first phase of nutrition. In Scolia dubia, as shown above, the two phases are distinct; one occurs within the terminal chamber, the other involves only the follicles.

Giardina says that the origin of the yolk ducts is in the unsevered protoplasm and cell membranes of the last differential cell-divisions which result in the formation of a "rosette" of nurse cells attached to an ovum or oöcyte. "L' origine delle connessioni tra l' oöcyte e le cellule nutrici e da ricercasi nella gia noto origine del rosette." Conditions in Scolia dubia indicate that this is a correct interpretation.

Conclusions.

1. The terminal filament does not take part in the supply of the primordial cells from which oöcytes and nurse cells are differentiated.
2. The follicle epithelium is not directly concerned with the nourishment of the ovum.
3. Nuclei within a cytoplasm to which they are exotic may divide amitotically.
4. There are two clearly defined stages of nutrition of the egg.

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Plate I.

Fig. 1. Terminal chamber. t. f., sections of terminal filament; a., degeneration space; e., egg cell; n., nurse cell; y. d., yolk duct; f. c., follicle cells. × 500.

Fig. 2. Two egg cells from distal end of terminal chamber. The lower one shows two nurse cells in a chain of yolk duct attachment. The nurse cells so attached to the egg cell do not indicate secretion activity. y. d., yolk ducts. × 1000.

Fig. 3. An older egg cell. The chromatin of the nurse cell having a yolk duct now shows a marked contrast with other nurse cells. v., vacuole. × 1,000.

Fig. 4. Later stage than preceding. × 1,000.

Fig. 5. The yolk duct about to break permitting the final nurse cell to enter the nurse follicle. ch., chorion. × 1,000.

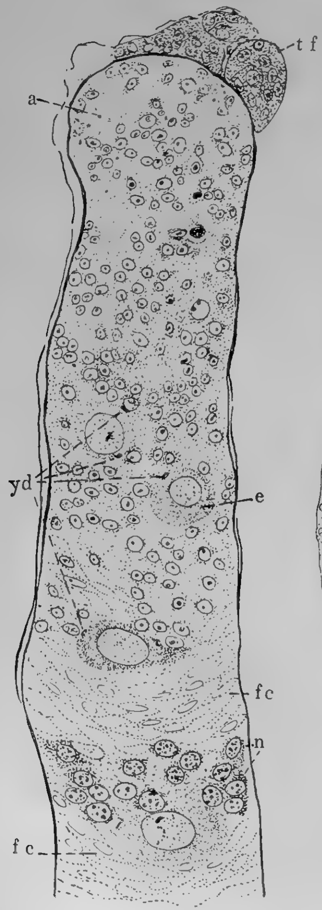
Fig. 6. Completed nurse and egg follicles. e. n., egg nucleus; m. n., migrated nuclei. × 500.

Fig. 7. Distal end of terminal chamber showing degeneration features, a. and b.; t. f., cells of terminal filament. × 1,000.

Fig. 8. Group of migrated nuclei. × 1,000.

Fig. 9. Amitoses of migrated nuclei. × 1,000.

Note.—Figs. 2-9 drawn with Zeiss camera lucida, Zeiss comp, eye-piece No. 6, Zeiss apochrom, 1.5. Figs. 1 and 10 were drawn with Bausch & Lomb camera lucida; B. & L. eye-piece No. 1 and 1-6 and 2-3 objectives.



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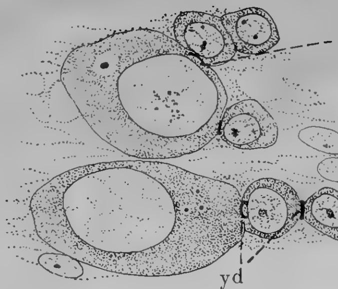

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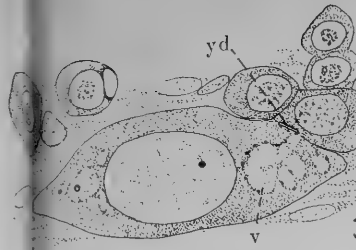

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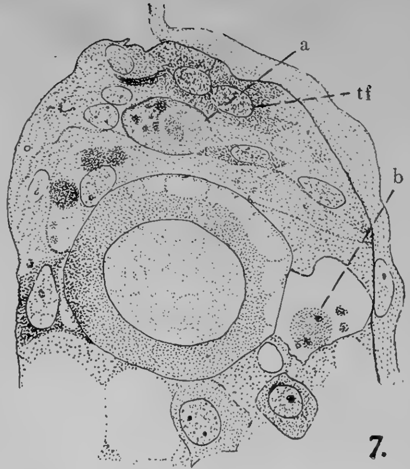

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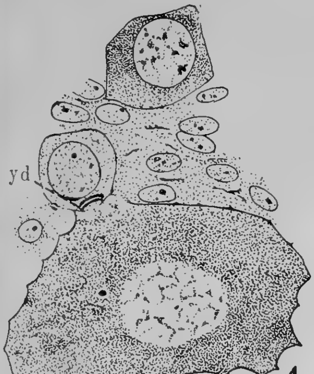

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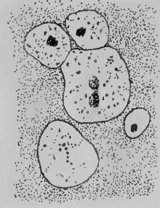
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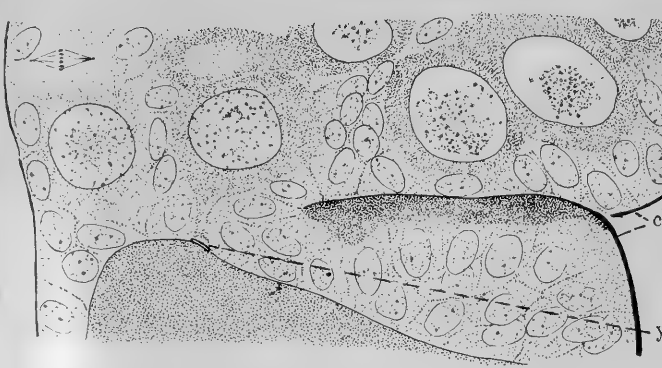

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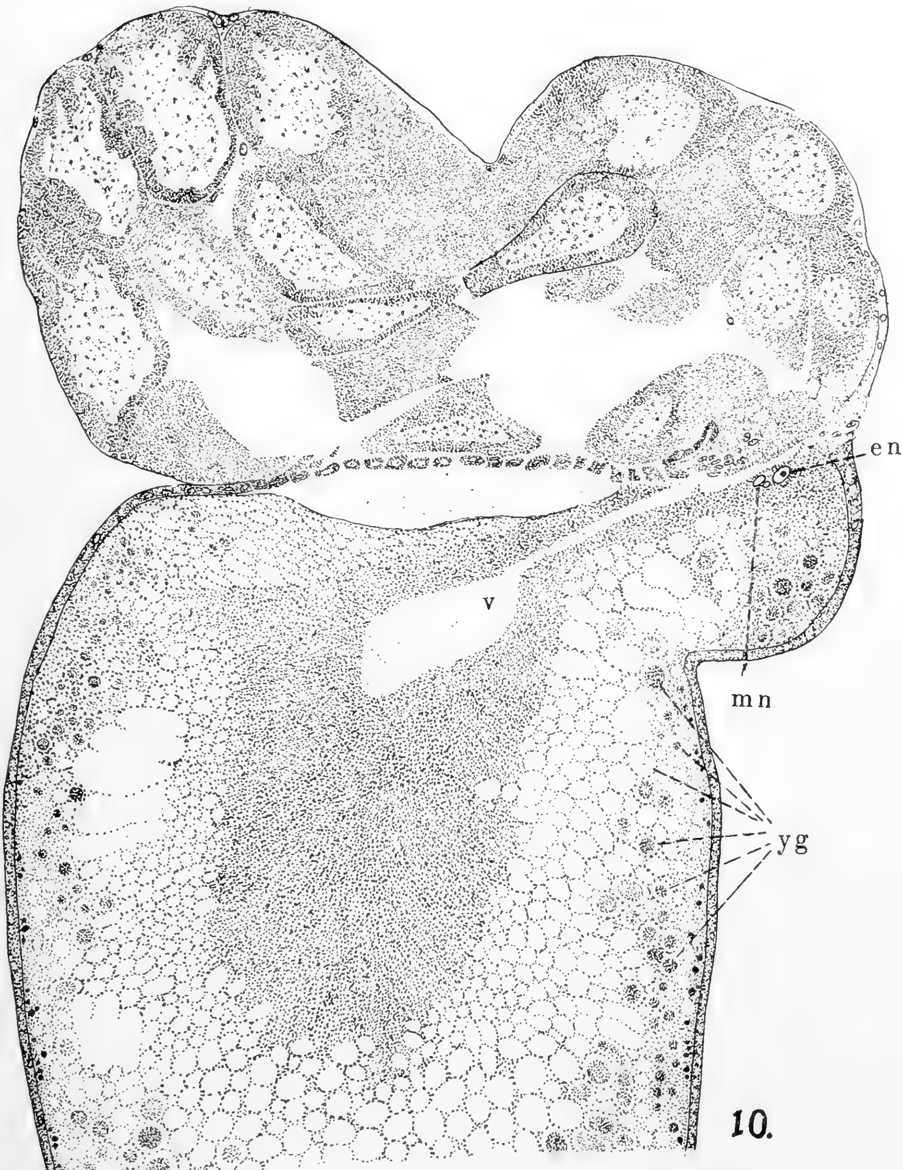
PLATE II.

FIG. 10. Late stage in the second nutrition phase of ovum. e. n., egg nucleus; m. n., migrated nucleus; y. g., yolk granules; v., vacuole. × 166.



10.

THE LATERAL NASAL GLANDS OF AMPHIUMA.

BY

INEZ WHIPPLE WILDER.

In describing the nasal region of Amphiuma tridactylum, H. H. Wilder '91b, mentions "eine laterale Drüsenmasse die ausserhalb des Cavum nasale liegt." This gland, he says, "bildet eine compacte ovale Masse und liegt subcutan in einer Vertiefung zwischen den Rändern des Nasale, Prämaxillare und Maxillare." He further adds, "so weit ich constatiren konnte, mündet die Drüse nach vorn durch zwei Ausführungsgänge in den Vorhof der Nasenhöhle ein." At Dr. Wilder's suggestion I began, several years ago, a study of the structure and development of this gland and a comparison of the gland with similarly located ones of other urodeles. This suggestion, however, led me incidentally to the study of certain peculiarities of structure in the nasal region of lungless salamanders (Plethodontidæ and Desmognathidæ), the results of which I have already published (Whipple '06); the results of the original line of research have, therefore, been deferred for treatment in this paper.

I. Description of the Structures in the Adult.

In the adult Amphiuma the lateral nasal gland is readily exposed by the removal of the skin and subcutaneous tissue from the dorsal surface of the head (Plate I, Fig. A). Superficially it has the appearance of a compound alveolar gland extending from near the tip of the snout about half-way back to the eye. It lies, as the above quotation indicates, wholly outside of the nasal capsule, lateral and slightly dorsal to it. It is bounded mesially by the nasal bone and latero-ventrally by the premaxillary and maxillary. The posterior portion of the gland, however, becomes partially enclosed between the maxillary bone and the cartilaginous nasal capsule.

From near the anterior end of the gland, in all of the specimens which I have examined, a single duct was found to extend over the posterior margin of the *fenestra rostralis* (Bruner's nomenclature) of the cartilaginous capsule, from whence it passes through a noticeable thickening in the wall of the nasal cavity, and opens upon its inner surface. I had the opportunity to study the same series of transverse sections of the adult head from which Wilder drew his conclusions above quoted, as to the existence of two ducts to the gland, and I examined these sections with especial reference to the relation of these ducts to the glandular mass. One duct could be definitely

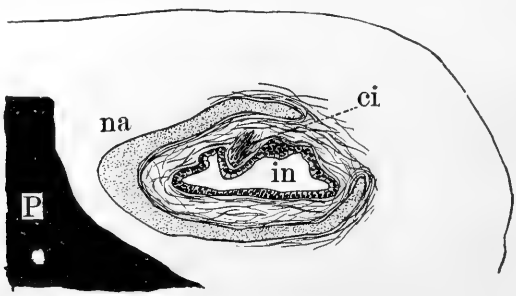


Fig. 1.—Transverse section through the introductory nasal passage of a small adult Amphiuma tridactylum. $\times$ 16 2/3. This section and those shown in Figs. 2, 3, and 4 are taken from the same series from which the reconstructions given on Plate I., Figs. B, C, and D were made. The locations of the sections are indicated upon Fig. D by lines correspondingly numbered. Abbreviations: ci, insertion of the constrictor naris muscle into the nasal epithelium; in, introductory nasal passage; na, nasal capsule; P, portion of the premaxillary bone.

traced to the alveoli of the gland; the second duct, however, when traced back from its orifice, which was slightly posterior and ventral to that of the first, was found to end blindly in close proximity to the glandular mass, but without actual connection with it (cf. Fig. 2, and Plate I, Figs. C and D, ad).

In connection with the opening of the duct of the gland the conformation of the nasal cavity must be understood. From the external naris the nasal passage extends at first somewhat mesially, then almost directly posteriorly for a short distance, the longer diameter of its lumen having in this region a horizontal position. In the

region where the duct of the lateral gland opens into it, however, the nasal passage makes an abrupt twist, so that the longer diameter of the lumen takes a vertical position (cf. Figs. 1 and 2). A short distance posterior to this point the olfactory epithelium begins, the portion of the passage anterior to this point serves, then, as an introductory passage comparable to that described by Bruner '01, and Seydel '95, in larvæ of various urodeles (e. g., Triton and Amblystoma), by Wilder '91a, in Siren, and by Hinsberg '01, in larval anurans and in urodeles.

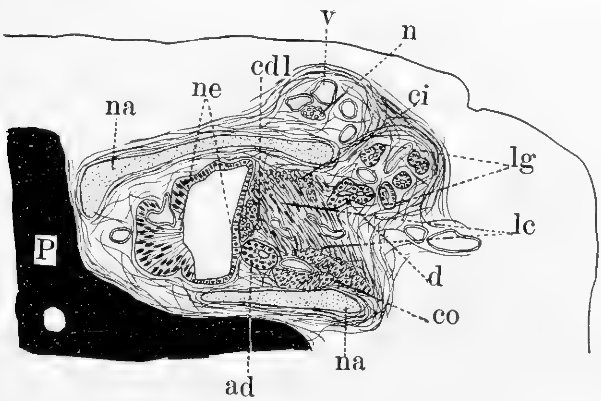


FIG. 2.—Transverse section somewhat posterior to Fig. 1. × 16 2/3. Abbreviations: ad, atrophied duct; ci, cross section of the constrictor muscle near its insertion; co, cross section of the constrictor muscle near its origin; cdl, cross section of the dilatator muscle; d, duct of the lateral nasal gland; lc, longitudinal section through the body of the constrictor muscle; lg, lateral gland; na, nasal capsule; ne, nasal epithelium; n, nerves; P, portion of the premaxillary bone; v, blood vessels.

The twisting of the nasal passage in its transition from introductory to olfactory regions involves a thickening in the wall of the passage. This thickening begins anteriorly in the mesial wall and, gradually increasing, extends spirally around the cavity, ending in the lateral wall. It results in the occurrence upon the inner surface of the cavity of a spiral fold or ridge following the course of the thickening. Upon the forward directed surface of this fold the duct of the lateral gland opens. Thus by the very conformation of the nasal passage the secretion of the gland is directed outward.

The spiral thickening in the wall of the nasal cavity is shown by histological examination to be composed of a mass of involuntary muscle fibers, which, although somewhat interlaced, reminding one of the relation of muscle fibers in the mammalian tongue, may be resolved into two distinct sets plainly to be identified as the *constrictor naris* and *dilatator naris,* which have been so fully worked out by Bruner '96 and '01, in various genera of the Salamandrida (cf. Figs. 1, 2, 3, also Plate I, Fig. C).

The *constrictor naris* of Amphiuma is especially well developed. It arises from the inner surface of the nasal cartilage near the

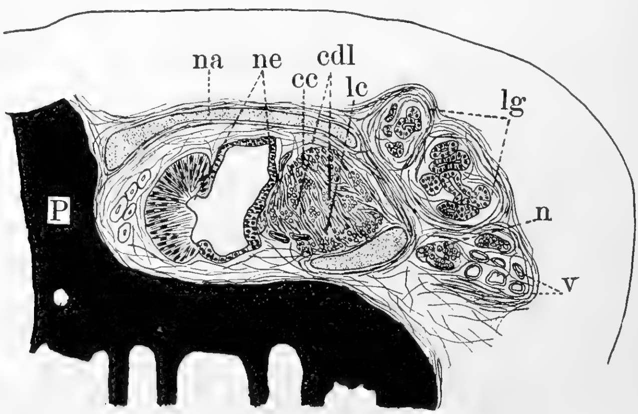


FIG. 3.—Transverse section posterior to Fig. 2. × 16 2/3. Abbreviations: cc, cross section of the constrictor muscle. Other abbreviations as in previous figures.

fenestra rostralis and extends first posteriorly and dorsally, then, after making a loop which partially encircles the nasal passage, it passes anteriorly and mesially to its insertion into the epithelium covering the dorsal and mesial portions of the spiral fold. The duct of the lateral gland lies in the concavity of this horseshoe-shaped muscle, the muscle itself constituting the thickening in the wall of the cavity through which the duct was described as passing. (Cf. Figs. 1, 2, 3, and Plate I, Fig. C.)

The second muscle, the *dilatator naris,* arises posterior to the loop of the *constrictor naris,* from the inner surface of the cartilaginous

capsule, and from the premaxillary bone, which upon the ventral side supplies the deficiency of the latter. From this origin it passes anteriorly to a somewhat extensive insertion into the nasal epithelium posterior and ventral to the opening of the duct. Many of its fibers appear, however, to end within the belly of the constrictor muscle.

The function of these muscles is very evidently the closing and opening of the nasal passage. The constrictor muscle, since its origin is anterior to its insertion, pulls forward and downward upon the spiral fold causing this fold to approach the opposite wall of the nasal passage, which at this point has a very narrow lumen. Moreover, the increase in thickness of the whole muscle mass incident to the very act of contracting causes the mass to press inward and forward, and thus helps to close the lumen. The dilator muscle, on the other hand, pulls posteriorly upon both the spiral fold and the belly of the constrictor muscle itself, and thus by its contraction opens the passage again.

The spiral fold is thus in structure and function like the crescentic fold which closes the external naris of the Salamandrida (Bruner '96 and '01), differing only in the fact that it lies, not at the external orifice, but at the inner end of the introductory passage. Because of this location, the movements of the spiral fold of Amphiuma cannot normally be observed, as can those of the crescentic fold of the salamandrids; however, in a living specimen which was in my possession there was a slight malformation of one external naris (probably due to an injury), so that the introductory passage was rendered funnel-shaped, and the movements of the spiral fold at the inner end of the funnel could be readily observed. As in the lunged salamandrids, the closure of the nasal passage of Amphiuma occurs during pulmonary respiration, and the function of the constrictor and dilatator muscles is undoubtedly associated with this act.

As to the gland itself, it presents superficially, as has been said, an acinous appearance (Plate I, Fig. D). The posterior portion particularly, shows a surface made up of numerous rounded eminences, very compactly massed together in definite lobes. However, serial sections through the gland, particularly in immature specimens, prove that it is fundamentally tubular in structure, but with modifi-

cations of the usual tubular type, of such a nature as to give the effect of an acinous gland.

Each lobe of the gland possesses a somewhat convoluted tubular axis, variable in diameter, but often of much larger size than the other tubules of the lobe. These central tubules possess a characteristically low epithelium and wide lumina (Fig. 4, *ln*); into them open, often in clusters but with apparently no regularity, many con-

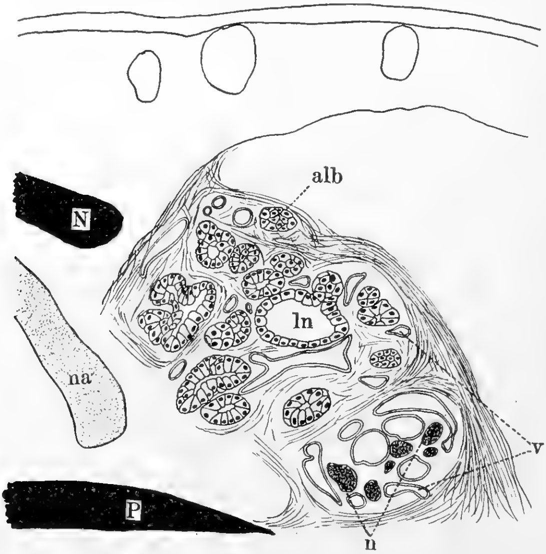


Fig. 4.—Detail showing transverse section of the lateral gland posterior to the section given in Fig. 3. Abbreviations: alb, somewhat isolated anterior lobe of the gland, sectioned through its extreme posterior end; ln, lumen of the expanded central tubule of one lobe of the gland; N, nasal bone. Other abbreviations as in previous figures.

voluted branches, each one of which, may in its turn exhibit much irregular branching. The branches often extend anteriorly as well as posteriorly from the point where they join the central tubule; at their convolutions there are usually acinous enlargements, and the smaller branches are frequently spherical in shape, thus forming true acini. It is the presence of these acinous enlargements and branches in the peripheral region that gives the acinous appearance to the gland. In contrast to the low epithelium and the wide lumina

of the central tubules of the lobes the branches are characterized by a tall columnar epithelium and, for the most part, very narrow lumina. They are evidently the actively secreting portions of the glands, while, judging from the large amount of coagulated material in the lumina of the central tubules, the latter serve as the reservoirs in which the secretion is held.

The central tubules of the various lobes gradually come together as they approach the orifice of the gland; but, so far as I have been able to determine, there is no single collecting tubule into which all the others open. The number is gradually reduced, however, by the confluence of the central tubules of the various lobes until a single one remains to make its way to the orifice as the duct of the gland. Although somewhat smaller in diameter than many portions of the central tubules, this duct has a large lumen lined with a low epithelium.

Several of my dissections and serial sections show a small anterior lobe, somewhat apart from the larger mass of the gland; this lobe connects with the duct of the glandular mass immediately posterior to the point where it bends to make its way across the constrictor muscle to its orifice. The method of development of the gland, to be discussed later, offers a possible explanation of the existence of this lobe.

A dense layer of connective tissue invests the entire gland while each lobe has a thinner investment. The gland is well supplied with blood-vessels. I have been unable to determine with certainty the innervation of the gland. A large nerve bundle, *ramus glandularis opthalmici profundi* II (Wilder), passes through a foramen of the maxillary bone, and issues in close proximity to the gland, in fact, the tubules of one lobe of the gland extend a short distance into this nerve canal. Although this nerve lies beside the gland throughout the remaining extent of the latter, I have been unable to demonstrate that any of these nerve fibers actually enter the gland. A branch of this nerve does, however, go to the spiral fold, and it is possible that some fibers of this branch innerve the gland also. The anatomical association of the gland and the nerve may, at any rate, be explained by the fact that the gland tubules in their development

Designation of series.	Length of specimen.	Length from tip of snout to eye.	Lateral gland tubules of right side.			Lateral gland tubules of left side.			Ratio of Length of whole glandular mass to length of head from the tip of the snout to the eye.	
			Number of tubules.	Relative location of orifices.	Length of tubules.	Number of tubules.	Relative location of orifices.	Length of tubules.	Right.	Left.
C. Trans. Larva.	30 mm.		0			0				
A. Trans.	60	1.84 mm.	1	(See Fig. 5, a.)	.465 mm.	1		.435 mm.	.25	.24
X. Trans.	78	1.98	2	Ant. and dorsal.	.210	3	Ant. and dorsal.	.105	.30	.295
				Post. and ventral:	.600		Middle.	.585		
							Post. and ventral.	.195		
							(See Fig. 5, b.)			
F. Long. Right Half.	125	2.5	2	(Undetermined.)			(See series G.)			
G. Trans. Left Half.	125	2.5		(See series F.)		3	Ant.	.120		.32
							Middle			
							Post.	.805		
B. Trans. Left Half.	150	2.835				2	Ant. and dorsal.	.375	.45	
							Post.	1.276		
							(See Fig. 5, e.)			
D. Trans. Left Half.	190	4.635		(See series E.)		3		1.86 (whole mass.)		.40
E. Trans.	190	4.635	2	Ant. and dorsal.	1.74		(See series D.)			.375
				Post. and ventral.	1.665					
				(See Fig. 5, f.)						

take the paths of least resistance, and would naturally work their way along the larger nerve bundles. A more probable source of innervation of the gland is from the *ramus glandularis opthalmici profundi* I, the branches of which spread out in the connective tissue of the snout dorsal and lateral to the gland; some of these fibers may be traced to the skin of the snout, but several intermingle with the anterior portion of the gland and seem to lose themselves within its mass.[1]

II. The Development of the Lateral Glands.

As the accompanying table shows, my study of the development of the lateral gland has been based upon serial sections of six specimens ranging in total length from 30 mm. to 190 mm. To this list may be added a single specimen 80 mm. in length in which the glands were studied by dissection. This latter method, while not absolutely trustworthy as to the exact relationships of the individual tubules, furnishes an extremely satisfactory corroboration of the results obtained by the more laborious method of reconstruction from serial sections. The dissections were made by slicing off with a sharp scalpel the lateral portion of the head of a specimen well hardened in alcohol, the cut passing obliquely through the nasal cavity in such a way that the whole of the external naris was included in the portion removed. This detached portion was then pinned out in a small dissecting pan and the work of exposing the gland was completed by dissecting from within the nasal cavity, since the only parts to be removed were the nasal epithelium and the cartilaginous capsule.

In the smallest specimen sectioned, a larva 30 mm. long, there is no trace of a lateral nasal gland or of nasal muscles.

The next stage, a 60 mm. specimen in adult form, shows a single tubular gland (Fig. 5 a) upon each side. Towards the posterior end, the lumen of the tubule becomes much enlarged and ultimately divides into two lumina, although in the external wall there is no evidence

[1]An article by Norris, '08, on the Cranial Nerves of Amphiuma has appeared since this manuscript left our hands, in which it is stated that neither of these branches innervate the lateral gland, and that the name *ramus glandularis* is therefore a misnomer.

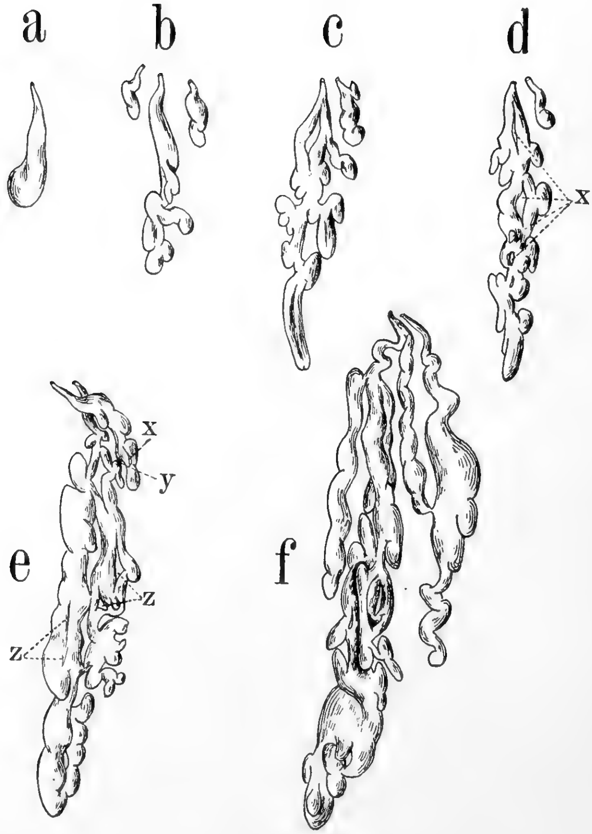


FIG. 5.—Developmental stages of the lateral nasal gland of Amphiuma. Although some of these are from the right and others from the left side, the drawings are so reversed as to give in each case the effect of a lateral view of the glands of the right side, *i. e.*, the dorsal region of the drawing is on the right hand side, and the ventral on the left. × 45. Abbreviations are explained in the text.

a. 60 mm. specimen, lateral view of gland of the right side, based on millimeter paper reconstruction from transverse serial sections.

b. 78 mm. specimen, lateral view of glands of left side (reversed), based on millimeter paper reconstructions from transverse serial sections.

c. 80 mm. specimen, internal view of glands of right side (reversed), from dissection.

d. 80 mm. specimen, internal view of glands of the left side, from dissection.

e. 150 mm. specimen, lateral view of glands of left side (reversed), based on reconstruction by the millimeter paper method from transverse serial sections.

f. 190 mm. specimen, lateral view of glands of the right side, based upon reconstructions by various methods. The posterior half of the larger gland possesses several tubules which are hidden from view.

of this division. The constrictor and dilatator muscles are already well developed and each tubule opens anteriorly into its respective nasal passage through a slender duct which has the same relationship to the nasal muscles as described above in the large adult.

A slightly older stage, 78 mm. long, shows a larger number of tubules and an increase in the complexity of structure of the gland. This specimen has two independent tubules upon the right side and three upon the left (Fig. 5 b), all opening in close proximity upon the anterior surface of the spiral fold. In each group the tubule having the most anterior and dorsal orifice is the shortest. In the group of three the middle one is by far the longest. All of the tubules exhibit convolutions, and the long tubule of each side has one or more branches.

An 80 mm. specimen studied by dissection shows very similar conditions on both right and left sides (Fig. 5, c and d). Here there are two independent tubules upon each side, one, the more ventral, being in each case much shorter than the other. The longer gland shows a considerable complexity of structure, the development of alveolar-like swellings, and the tendency toward a longitudinal splitting of various regions being noticeable features. The result of the latter process is the very curious condition in which the whole gland appears to be splitting longitudinally into two main parts. Thus in d four regions indicated by x show complete separation, while the other parts still remain in communication.

A 125 mm. specimen shows upon the right and left sides two and three tubules respectively. Upon the right side the more dorsal gland is the extensive, complicated one; upon the left side the middle and the ventral (posterior) one form a complicated glandular mass, while the more dorsal tubule is short. The series is not sufficiently perfect to allow one to work out accurately the course of the middle and ventral tubules. As nearly as I can determine, however, the middle one contributes by far the longer and more complicated portion of the glandular mass.

One side only, the left, of a 150 mm. specimen was sectioned (Fig. 5, e). In this there are two independent glands; the shorter one has the more anterior and dorsal orifice, but it bends about in such a

direction that the larger portion of the gland lies ventral to the larger gland. This shorter gland is somewhat convoluted and has one branch; it exhibits several alveolar distensions and in one region (x) is split for a short distance into two tubules, which communicate with each other posteriorly as well as anteriorly. At one point (y) this gland lies in such close proximity to one of the branches of the larger gland as to suggest that a secondary intercommunication is about to arise between two originally independent tubules, a condition which was actually found to exist in a still more advanced stage.

The larger gland in this specimen shows a great advance in complexity of structure. Marked distensions and convolutions occur throughout the gland. The splitting of the entire gland into two parts, already well begun in the 125 mm. specimen, is here almost completely accomplished, while secondary longitudinal splittings seem to be in progress in other regions (z). Many parts show not only alveolar swellings at points of convolutions, but the formation of true alveolar branches. The extreme posterior end shows a peculiar doubling of the gland upon itself, a condition possibly arrived at by a simple convolution, but more probably by an incomplete longitudinal splitting.

The condition shown by a 190 mm. specimen indicates a rapid advance of the developmental processes. Here the right side (Fig. 5, f) shows two glands. The shorter (ventral) one is convoluted and possesses an enormous lumen throughout the middle third of its course; although by tracing the tube anteriorly, its connection with the epithelium of the introductory nasal passage may be demonstrated, its anterior portion seems to be in a state of atrophy and consists merely of a slender cord of cells with no trace of a lumen. However a secondary communication with the nasal passage has been established in that the distended middle region of the tubule joins a slender branch of the larger gland, to the complicated mass of which it has thus become annexed.

The larger gland has a slender duct which divides almost immediately into two tubules, one being the relatively simple and short one which, after making a few convolutions, communicates at its terminus with the distended portion of the other gland as above described;

the other makes a few abrupt convolutions and then divides into three subdivisions. Of these two are hardly more than simple, slightly convoluted tubules which have not more than half the length of the third (and middle) division. The latter at about the middle of its course becomes suddenly greatly complicated, showing convolutions, distensions, longitudinal splitting, branching and alveolar swellings, and is apparently dividing up into the anlagen of the lobes of the posterior half of the adult gland, while the anterior half will be formed from the other shorter branches.

The left side of the same specimen possesses a glandular mass equal in extent to that of the right side. This opens into the nasal passage by three separate orifices, but, owing to the fact that the series is somewhat imperfect, it is impossible to trace out the relationship of the individual tubules.

A comparison of the above facts shows that the lateral nasal gland and the nasal muscles with which it is associated are structures characteristic of adult life. The gland makes its first appearance as two or three closely associated evaginations of the lateral wall of the nasal cavity near the inner end of the introductory nasal passage, while the muscles arise from the connective tissue underlying the nasal epithelium immediately posterior to this point.

That the development of the tubules takes place with great rapidity is shown, not so much by comparison of the proportionate length of the glandular mass in the successive stages studied, although this comparison shows on the whole a gradual increase, as by the rapidly increasing complexity of structure of the tubules. Moreover, one of the tubules (the middle one when there are three) undergoes a development so much more rapid and complicated than the others that I shall designate it the main gland of the group. In its development several distinct processes occur. The tubule shows an early tendency to become convoluted, often making abrupt curves. Further, there is much branching not only by the simple process of evagination, but even more commonly by the longitudinal splitting of a distended portion of a tubule into two tubules which remain in communication at one or more points. All of this branching seems to be in its details quite irregular, although carrying out a certain underlying plan of

development which results in laying down the anlage of a glandular mass consisting of many compact lobes. As development continues, the tubular nature becomes more and more masked by the appearance of alveolar distensions; these appear first at the numerous bends in the tubules and at the termini of the branches, and finally by a direct formation of alveolar outpushings from the sides of the main tubules. The resulting compact glandular mass thus acquires a pseudo-alveolar structure, consisting of many complicated lobes, each with a central tubule.

In this process of development there occurs a gradual differentiation of epithelial regions. Only the anterior portion, the duct of the gland, retains the original low cubical epithelium which is characteristic of the entire gland in its early stage. The remaining portions of the glandular regions first seem to undergo throughout a gradual change to a columnar form of epithelium. Then with the development of regions with large lumina, the anlagen of the central tubules of the adult gland, the epithelium of these distended regions tends to become cubical again while the smaller branches retain their columnar form of epithelial cells.

While the main tubule is undergoing this complicated process of development, the other tubules of the group are passing through a more restricted development, often failing to exceed the condition of simple tubules. They show, however, in their development, the same tendencies that the main gland exhibits and in some cases become somewhat complicated.

As to the fate of these accessory tubules, we find in the example given in Fig. 5, f, one case where an accessory gland which has attained a considerable size, becomes, by means of a secondarily established communication, a part of the main glandular mass, while at the same time its own duct suffers atrophy. It is possible, of course, that accessory tubules may sometimes persist as independent glands; but inasmuch as in no case have I found more than a single functional duct in any large adult specimen, I am of the opinion that the more usual course is for the ducts, at least, of these accessory glands to atrophy, the process extending also, possibly, to the glandular portion when this is small, while those which arrive at a considerable

size (perhaps generally one in each set) become annexed to the main gland. In this process of annexation we have an explanation of the somewhat isolated anterior lobe of the adult gland already alluded to (Plate I, Fig. D, alb), a lobe which probably arises separately and only secondarily becomes connected with the main gland. The atrophy of another accessory duct explains, also, the existence in this case of an additional short duct (ad) ending blindly with no connection with the glandular mass.

This atrophy of ducts of accessory tubules and their secondary connection with the main gland indicates a physiological demand for concentration of the secretion at a single point; on the other hand, there should be mentioned, as a possible cause for this atrophy, the fact that a Trematode parasite (as yet unidentified) is frequently found lodged in close proximity to, or even within, the lucts of the glands. This parasite is not confined to this region, for it has been found lodged in the muscles of various parts of the head, and in the connective tissue underlying the skin and the epithelium of the mouth and nasal cavities. So far as I know, the structure and life-history of this Trematode have not been worked out. It is possible, however, that the ducts of the lateral gland are vulnerable points through which the parasite frequently gains entrance; the presence of such a parasite within or near one of the ducts would be very likely to cause its atrophy, while the glandular region of the tubule would establish a secondary communication with the exterior through a neighboring branch of another tubule and would thus continue its functional activity.

III. The Homology of the Lateral Nasal Gland and Its Associated Structures.

In both the Salamandrida and the Anura there are certain tubular glands which open in close association with the external naris and are known as the external nasal glands. The number of these glandular tubules associated with each naris varies in different species from two to fifteen. These structures have long been recognized and their relationships have been worked out in various species by Wiedersheim '76, Seydel '95, Riese '91, Born '76, and Bruner '96 and '01.

The latter author has not only described the glands themselves but has shown that they have a definite association with the constrictor and dilatator muscles of the external naris, since the ducts of the glands pass through the loop of the former muscle. Moreover, he emphasizes the importance of this anatomical relationship by showing that in Triton and Amblystoma (the genera in which he investigated the development of these organs) the muscles and glands arise simultaneously, the former from the connective tissue in the walls of the larval introductory nasal passage, the latter by evaginations of its epithelial lining; and a similar mode of origin which he finds in the Anura further establishes the general homology of these structures.

In an article already referred to (Whipple '06) I have shown that the external nasal glands of the Desmognathidæ and Plethodontidæ belong to a series of tubular glands which I have collectively termed the naso-labial glands; some of these, the external nasal glands, have their orifices near the margin of the naris, the remainder open along the border of the naso-labial groove, a structure which is peculiar to these lungless forms and extends from the latero-ventral angle of the naris to the edge of the upper lip. Many of the naso-labial glands are short, simple tubes; a few, however, particularly those associated with the naris itself, attain enormous proportions in their development, often extending even posterior to the orbit. Moreover, they become much complicated by the formation of branches and alveolar convolutions. Their great extent and complexity has been shown by Wiedersheim, but seems to have been overlooked by Bruner, who describes the external nasal glands in general as exceeding but little in extent the *fenestra rostro-lateralis* of the cartilaginous nasal capsule.

Ontogenetically the naso-labial glands of Desmognathus make their first appearance in the larva as it approaches its transformation to the adult stage, at the time when the only indication of a naso-labial groove is a slight ventral prolongation of the external nasal orifice. At this time two glands appear, the first an evagination of the epithelium at the inner edge of the incipient groove, the second a lateral evagination of the lining of the introductory nasal passage. As

development continues, the groove becomes prolonged ventrally by an infolding of the epidermis, and other glands make their appearance along its mesial border. In a large adult I have found as many as twelve glands connected with a single naris and its groove.

Comparing, now, these naso-labial glands of the Desmognathidæ and Plethodontidæ and the external nasal glands of other salamandrids with the lateral nasal gland of Amphiuma, it is very evident that we are dealing with homologous structures. This homology is shown by the time of origin of the lateral gland during transition from the larval to the adult form; by the derivation of the gland from the epithelium of the introductory nasal passage; by its close association, not only in location but in time of origin, with the constrictor and dilatator muscles; and finally, by its first appearance, not as a single gland, but as a group of several distinct tubules. In fact those developmental changes which result in the final pseudo-alveolar nature of the gland in Amphiuma are foreshadowed by the conditions shown by many of the salamandrids. The chief difference in anatomical relationships between the lateral gland of Amphiuma and the external nasal glands of other forms is in the location of the glandular orifice within the nasal passage rather than upon its margin. This difference is evidently due to the retention of the introductory nasal passage by the adult Amphiuma, whereas in other forms it is a larval organ and disappears in transition into the adult form. Thus the inner end of the introductory nasal passage of Amphiuma is the real homologue of the external naris of the Salamandrida, and the spiral fold which closes the lumen at this point corresponds to the crescentic fold which closes the external naris of other forms.

We come now to the question of possible homologies among the other Derotremata and the Perennibranchiata. The tubular glands of Proteus, described by Oppel '89, offer some interesting points for comparison with the external nasal glands of Salamandrida and the lateral nasal gland of Amphiuma. Oppel apparently examined four specimens of Proteus in regard to this point. The facts given in his description of these four individuals I have tabulated as follows:

Specimen.	Length.	No. of Tubules: Right.	No. of Tubules: Left.	Length of Tubules: Right.	Length of Tubules: Left.
	mm.			mm.	mm.
1	114	1	1	0.6	0.87
2	125	2	1	0.57 (medial) 0.83 (lateral)	0.48
3	215	6	5	0.63–0.84 (four shortest tubules)	0.315 (longest of the three short tubules)
				1.545 (the longest of the lateral group of three)	1.303 (the longer of the lateral group of two)
				2.535 (middle)	1.335 (middle)
4	121	3	3	0.21 (medial) 0.6 (lateral) 2.835 (middle)	0.285 (medial) 0.945 (lateral) 3.09 (middle)

From these statistics we see that the number of tubules upon one side varies from one to six, the number upon the two sides of one individual being, however, approximately equal. Moreover, the tubules, where more than one are present, vary much in length, as do also the longest and the shortest tubules of different individuals. Thus the range of length in three individuals of approximately the same size (Nos. 1, 2 and 4) is from 0.21 mm. to 3.09 mm. and the extremes are found in the same individual. The longest tubules in these three individuals vary from 0.83 mm. to 3.09 mm. It is noticeable, moreover, that when there are only two tubules the more lateral is the longer; while in a set of three or more the longest one is the middle one (or one of the middle ones) of the group. For example, the set of six on the right side of specimen No. 3 is disposed in three groups, the medial group consisting of two short ones, the lateral group of three, two short and one much longer, while between these two groups lies the middle tubule which is the longest of the six. There is a noticeable symmetry in the approximate length of the tubules on the two sides of an individual. Although the shortest specimen (No. 1) has the smallest number of tubules, and also

relatively short ones, it does not appear from the whole set of observations that the variation either in number or in length of tubules is in any way proportionate to the size of the individual (cf. Nos. 2 and 4).

In the single specimen of Proteus examined by me (a series of transverse sections of an adult head, length of specimen unknown) there are two tubules upon the right side and three upon the left. Of the group of three the lateral is the shortest and measures 0.3008 mm.; the medial one measures 1 mm., and the middle one of the group 1.203 mm. There is, however, a disconnected tubule ending blindly at both extremities, about 1 mm. long and beginning 2.106 mm. posterior to the blind end of the middle (longest) tubule, Assuming that this detached portion was originally connected with the longest tubule and that from some cause the intermediate region has atrophied, the total length of this tubule would be $1.203 + 1.0 + 2.106$, or 4.309 mm. The two tubules on the right side measure respectively 3.008 (medial), and 3.759 (lateral).

As to the orifices of the tubules my observations practically agree with those of Oppel, who says "Diese ist für alle gemeinsam die Stelle, an welcher die äussere Nasenöffnung, d. h. der von einem niedrigen Plattenepithel ausgekleidete Vorraum ausserhalb der mit dem Riechepithel gekleideten Nase an die äussere Haut angrenzt. Von der hintern Seite dieser Oeffnung entspringend ziehen die Canäle zum Theil medial, zum Theil lateral unterhalb der im Bogen nach hinten steigenden Nasenhöhle gleichfalls nach hinten, um, ohne sich zu verzweigen, blind zu endigen."

Oppel offers no theory as to the homology of these glands. He assumes, however, that the longest tubule, which in one specimen extends nearly to the eye, is the "Thränencanal," a conclusion which has been overthrown by the later researches of Born '76. So far as I know, the development of these tubules has never been studied, but their general structure and location show that they are homologous with the lateral gland tubules of Amphiuma and with the external nasal glands of other amphibians. A point which would, at first, seem important in the establishment of this homology is, however, lacking in that, unlike the other forms in which external nasal glands are present, there is no trace in Proteus of either con-

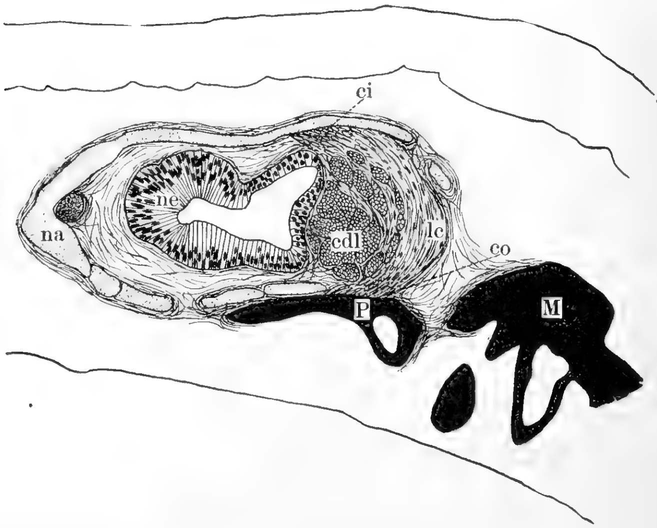


FIG. 6.—Transverse section through the anterior nasal region of an adult Cryptobranchus allegheniensis. Abbreviations as in previous figures.

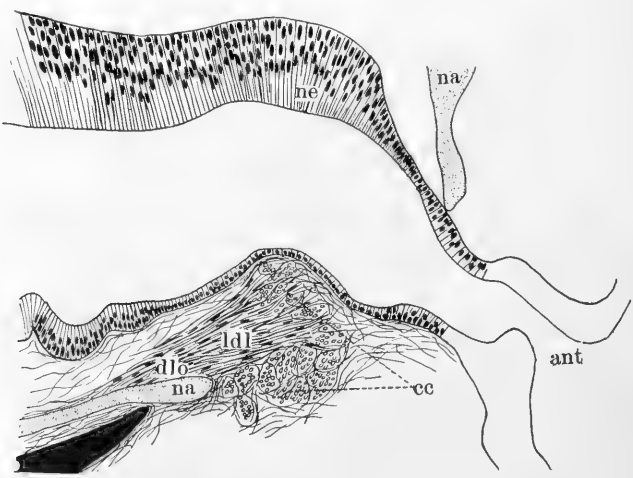


FIG. 7.—Frontal section through the anterior portion of the nasal cavity of an adult Cryptobranchus allegheniensis. Abbreviations: ant, external naris; cc, cross section of the constrictor naris muscle; dlo, origin of the dilatator muscle; ldl, longitudinal section of the dilatator muscle. Other abbreviations as in previous figures.

strictor or dilatator nasal muscles. On the other hand, the extensive range of variation in both the number and the length of the gland tubules, as well as the actual evidence of atrophy which was found in the case of one of the tubules, indicates that the glands have lost their functional importance in Proteus, and that they are in a more or less degenerate state. If, now, this function was originally associated with that of the constrictor and dilatator muscles, the failure of the latter in Proteus is quite consistent with the condition of the glands. The absence of the muscles and the degenerate state of the glands are both facts which will prove of importance in the final consideration of the functions of these parts.

As to the other perennibranchs, in both Necturus and Siren there is complete failure of both the external nasal glands and the constrictor and dilatator muscles; and Axolotl, if indeed this permanent larva is to be considered in this group, is described by Bruner as possessing the rudiments of both glands and muscles, but, as is the case with other salamandrids, these do not appear until the animal has nearly arrived at the adult stage. Typhlomolge, another form which has been classed with the perennibranchs, was shown quite conclusively by Miss Emerson, '05, to be a permanent larva of some lungless form, probably closely related to the genus Spelerpes. In view of this fact, one would hardly expect to find external nasal glands or constrictor and dilatator muscles except, possibly, in the very rudimentary condition which is characteristic of that stage of the larva immediately preceding the transition into the adult form. As a matter of fact, the single series of sections through the head of a Typhlomolge which, through the kindness of Miss Emerson, I had the privilege of examining, showed no evidence whatever of any of these structures. Too much dependence should not be placed upon this observation, however, as the specimen from which the series was made was somewhat imperfect in the region of the external nares.

Cryptobranchus alleghenicnsis possesses well developed constrictor and dilatator muscles (Figs. 6 and 7), as may be readily demonstrated either by dissection or by microscopic sections; I have, however, been unable to find the slightest trace of any external nasal glands.

To summarize, among the lower urodeles Proteus and Amphiuma alone possess the homologues of external nasal glands, while the occurrence of nasal constrictor and dilatator muscles is limited to Cryptobranchus and Amphiuma.

IV. The Function of the Lateral Gland.

Because of the apparently constant anatomical and developmental association of the external nasal glands with the constrictor and dilatator muscles of the naris in the salamandrids, Bruner drew the logical conclusion that there is also an intimate physiological relationship between these structures. Following out this line of reasoning, he concluded that, as the function of the muscles is to alternately close and open the external nares during the act of pulmonary respiration, the glands, the orifices of which are upon the margin of the nares, probably serve the function of lubricating the edges of the crescentic fold to insure tight closure; and he suggested, further, the probability that the mechanical device by means of which the secretion is discharged is the pressure exerted upon the gland tubules by the contractions of the constrictor muscle which closes the naris.

With regard to the embarrassment to this theory presented by the lungless salamandrids, in which the entire apparatus, both glands and muscles, is found, although, of course, there is no pulmonary respiration, Bruner suggested that the function of closing and opening the nares must here subserve some other purpose, such as, for example, the exclusion of foreign substances, particularly water, from the nasal passages. He thought the muscles in these forms less strongly developed than in the lunged forms, and apparently did not recognize the extreme degree of development which the glands have attained in the lungless species.

My own recent studies of the breathing habits of both lunged and lungless salamandrids (Whipple, '06) have led me to believe that the function of excluding water and other foreign substances from the nasal passages is the more generalized and therefore the primary function of the nasal muscular apparatus. The muscles are on the whole about equally well developed in both lunged and lungless

forms. Moreover, the contact of any foreign body with the snout is followed at once by the closure of the nares. As a general means for protecting the delicate nasal epithelium from dirt and other foreign matter in forms such as Desmognathus and Plethodon, which are burrowing in habit, this device must be invaluable. Further, in all the lungless forms (Plethodontidæ and Desmognathidæ) I have found that the exclusion of water from the nasal passages is an absolutely fixed habit, so much so that even when they are forced to remain under water for days, the nares are kept closed and aquatic bucco-pharyngeal respiration, such as occurs in the lunged forms under similar conditions, is never established. So great is the importance of this exclusion of water from the nasal passages, that in connection with each external naris a highly specialized device, the naso-labial groove, has developed, which acts as a gutter through which the tiny drop of water which naturally lodges in the nasal depression is drained off before the naris opens, and is thus prevented from being drawn into the nasal passage.

Even in the case of the lunged salamandrids a temporary submersion is accompanied by closure of the nares, and thus the animal is spared the physical inconvenience incident to a change of respiratory medium, although a prolonged stay in the water involves, in all the lunged species that I have experimented with, a transition from aerial to aquatic bucco-pharyngeal respiration.

Undoubtedly this device for excluding foreign substances from the nasal cavities has become of use in pulmonary respiration as a means for preventing the escape of air during that phase of the respiratory act when the mouth is used as a pump to force air into the lungs. But that closure of the nares for this purpose is not essential is shown by the fact that Necturus, Siren, and Proteus all effect pulmonary respiration in the absence of these muscles, and frequently (in Necturus at least) with the escape of air through not only the nares, but also the gill slits. Even in Amphiuma, in which the nasal muscles are present, there is frequently some loss of air through the gill slits during the act of filling the lungs. This escape of air can be readily observed in both Necturus and Amphiuma when the animals are in the water. In the case of lunged salamandrids, also, I

have several times noted that the closure of the nares during the act of filling the lungs is not perfect, although usually it is so.

It should be noted that the function of the nasal muscles is primarily associated with the attainment of a terrestrial mode of life. Even Cryptobranchus allegheniensis may not prove to be so decided an exception to this statement as would at first appear in view of its aquatic mode of life. I have not had the opportunity to study the function of the nasal muscular apparatus in living Cryptobranchus. Smith '07, reports however, that this species is somewhat burrowing in its habits, and thus its power to close the nares must at such times be of great value. Further, the species shows tendencies toward adaptation to terrestrial life. The streams in which it lives are liable to become almost dry. I have known specimens to live several days entirely out of water during transportation, not even surrounded by a wet packing, and to be normal and active at the end of the journey. Since the systematic position of Cryptobranchus is more or less of a problem, it is even possible that the presence of the nasal muscles may be due to an ancestral terrestrial form.

As to the external nasal glands, their function also seems to be a more generalized one than that suggested by Bruner, namely, the lubrication of the edges of the nasal orifice to insure tight closure during respiration. There is in terrestrial air-breathing forms a great necessity for some device for keeping the thin delicate skin which covers the crescentic fold moist and pliable; for this skin is, owing to its location, constantly exposed to the drying effects of the air as it moves in and out of the nasal cavity during bucco-pharyngeal as well as pulmonary respiration. The acinous glands which are abundantly developed for moistening the skin in other regions, are wholly lacking here, probably because their large size would require a greater thickness of skin than is consistent with the necessary flexibility; but these external nasal glands with their large extent of secreting surface are so deeply embedded that they cannot interfere with the free movements of the skin, and their secretion is discharged through slender ducts upon the surface of the fold, thus keeping the region so thoroughly moist as to counteract the excessive drying effect incident to the location.

The larger number and greater extent of such tubules in Amblystoma as compared with their very limited development in secondarily aquatic forms such as Diemictylus emphasizes the association of their function with aerial respiration under terrestrial conditions; and their complete absence in the aquatic forms, Necturus, Siren, and Cryptobranchus, adds further corroboration. That the secretion of these glands, or of their homologues, may come to subserve, secondarily, a more specialized function, I have shown elsewhere in the case of the naso-labial group of glands of the Plethodontidæ and Desmognathidæ, but the primary function seems certainly to be this more generalized one of protecting the delicate skin of the crescentic fold from the drying effects of the atmosphere.

Here Proteus seems to be an exception, for it is a wholly aquatic form which possesses external nasal glands. Their rudimentary nature must be remembered, however. This fact shows that whatever the environmental condition may have been to which the glands were an adaptation, this condition no longer exists. If, as seems probable, this condition was one of terrestrial life, the present form must be looked upon not as primitive, but as either a degenerate form or a permanent larva. The absence of nasal muscles is quite in accord with either view.

Turning now to Amphiuma, we find ourselves confronted by the problem of external nasal glands which have not only reached a high degree of complexity of development, thus bearing witness to the importance of their function, but which in their development have concentrated their secretion at a single point, although they begin their development as do the external nasal glands of other urodeles as several separate tubules. To explain the special function which this glandular mass has in Amphiuma I have considered in what respects the needs and adaptations of Amphiuma differ from those of other urodeles, and have directed my observations along those lines.

In habit the Amphiuma is terrestrial as well as aquatic. Even under aquatic conditions it spends much of its time burrowing in the mud at the bottom of the water; and in its terrestrial habitat it is described as living in the soft mud and burrowing through it almost like an earthworm. The whole form and structure are well suited

to this environment; the attenuated body, reduced legs, and prolonged pointed snout are evidently adaptations to such a mode of life. Moreover, it is terrestial in its egg-laying habits. O. P. Hay '88, gives an interesting account of his observations of a large female Amphiuma found guarding a mass of eggs at a considerable distance from any water. Among other observations of this specimen, he calls attention to the fact that the overlapping and interlocking lips afford a very tight closure of the mouth against dirt while the animal is burrowing, and that the gill slits are also capable of tight closure against the entrance of foreign matter.

Table Giving the Proportions of the Snouts of Various Urodeles.

	Ratio of length of snout to width of head from eye to eye.
Triton alpestris	.82
Diemyctylus viridescens	.75
Desmognathus fusca	.87
Cryptobranchus allegheniensis	.59
Amphiuma tridactylum	1.21

Not only, however, is the mouth thus perfectly protected, but, through the action of the constrictor muscle of the naris, the delicate nasal epithelium is also protected from the entrance of dirt. It will be remembered, however, that the spiral fold which is drawn across the nasal passage by the contraction of the constrictor naris muscle is located not at the external orifice, but at the inner end of an introductory passage. Undoubtedly the persistence of this passage, which in the salamandrids seems to be a larval organ disappearing with the transition to the adult form, is correlated with the enormous elongation of the snout as an adaptation to burrowing. The relative proportions of the heads of various urodeles are shown by the indices in the accompanying table, which serves well to emphasize the peculiarly

elongated snout of Amphiuma. This adaptation, however, involving as it does the persistence of the introductory passage, possesses the disadvantage of affording no protection against the entrance of dirt into this portion of the nasal passage, while, on the other hand, the very pressure incident to the act of burrowing tends to force the dirt into these introductory passages. Moreover, this lengthened passage gives an additional area exposed to the drying effects of air as it passes in and out with the respiratory act when the animal is out of water and is not actually burrowing. Here we have a clue to the function of the lateral or external nasal gland. The single orifice through which the gland discharges is located, as has been said, upon the forward directed surface of the spiral fold. The secretion is therefore, by the very conformation of the parts, directed outward. Thus during the respiration of air, in the long journeys which these animals are said to make under the loose leaves and sticks covering the ground of the swamps which they inhabit, the copious secretion must serve its primary function of keeping the lining of the introductory passage from drying; and when the animal is burrowing the secretion is undoubtedly used as a means for flushing out the fine particles of dirt which tend to plug up the introductory passage; but for some such device as this the dirt would, with the reopening of the inner end of the introductory passage and the resumption of respiration, be drawn into the nasal cavity and thus defeat the purpose of the constrictor muscle.

This latter function of the gland I was able to demonstrate experimentally by first carefully drying with filter paper the whole external nasal region of a living Amphiuma, and then filling the introductory passages with dry dirt. After a few moments the dirt became very moist and was soon forcibly expelled, leaving the passage quite clear and clean. A forcible expiratory act seemed to assist the glands in their final effort at expelling the obstruction.

Thus we see both in the very strongly developed condition of the constrictor and dilatator muscles and in the highly specialized state of the external nasal glands of Amphiuma further adaptations to the burrowing habit which in other respects has had such a decided effect upon the form and structure of the animal.

Smith College, Northampton, Mass., July 20, 1908.

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ABBREVIATIONS.

ad, atrophied duct of lateral gland.
alb, somewhat isolated anterior lobe of gland.
ant, external naris.
c, constrictor muscle.
ci, insertion of constrictor into the nasal epithelium.
co, origin of constrictor, cut from the inner surface of the nasal capsule.
d, the duct of the lateral gland.
dl, the dilatator muscle.
dlo, the origin of the dilatator muscle cut from the inner surface of the nasal capsule.
fr, fenestra rostralis.
g, lateral gland.
in, introductory nasal passage.
M, maxillary bone.
N, nasal bone.
na, nasal capsule.
ne, nasal epithelium.

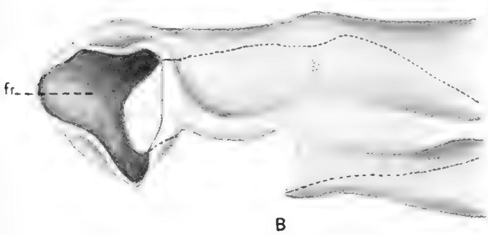
EXPLANATION OF PLATE I.

Fig. A. Dissection of snout of adult Amphiuma showing the relation of the laternal or external nasal gland to surrounding parts.

Fig. B. Drawing, based on a millimeter paper reconstruction, showing a lateral view of the anterior portion of the left cartilaginous nasal capsule of an adult Amphiuma.

Fig. C. Drawing, based on a millimeter paper reconstruction, showing the relationship of the constrictor and dilatator muscles to the walls of the introductory nasal passage of an adult Amphiuma, left lateral view.

Fig. D. Drawing, based upon a millimeter paper reconstruction, showing the anterior nasal region of Amphiuma, lateral view of left side.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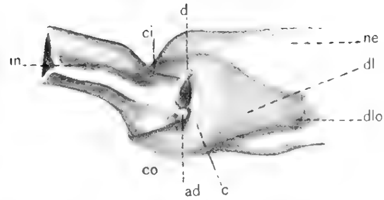
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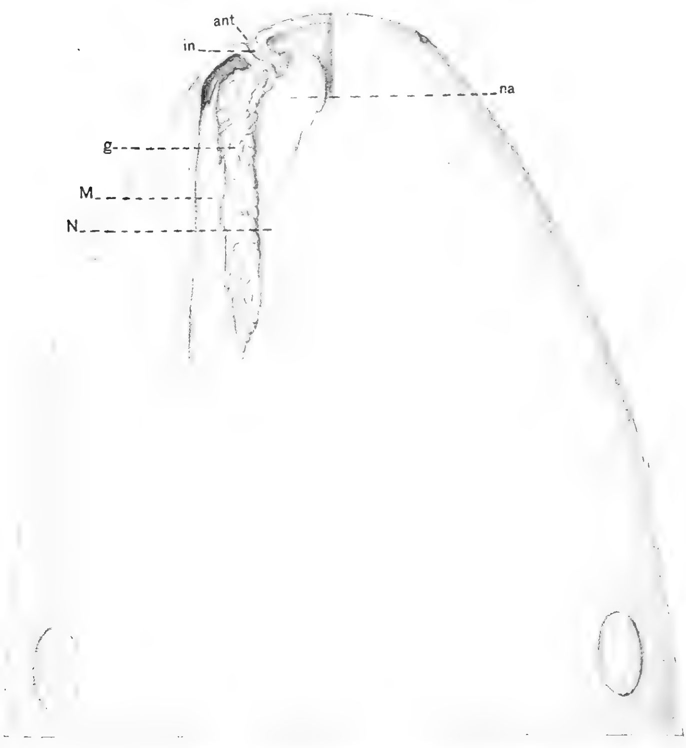
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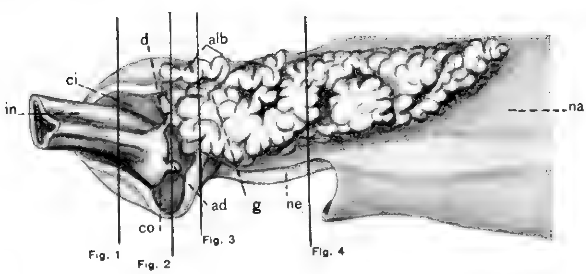
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THE CILIUM AS A KEY TO THE STRUCTURE OF CONTRACTILE PROTOPLASM.

BY

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Contribution from the Biological Laboratory of Clark University.

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Introduction: Statement of Problem.

A complete title for this paper would be: The Cilium studied comparatively as a test of microscopical methods and a key to the structure of contractile protoplasm.

The attempts of recent investigators to resolve the activities of contractile structures into phenomena due to alterations in the surface tension of a complex fluid are proving as unsatisfactory as the older attempts to identify contractility with chemical and electrical processes. Those who approached the problem from this standpoint—Berthold, Quinckne, Bütschli, Rhumbler, Jensen and others—used the Amoeba with its activities as "Ausgangspunkt" for their researches. Unfortunately they did not determine the real character of the movements here, and, therefore, their theories of protoplasmic movement based on the supposed activities of the protoplasm of the Amoeba have little value. The work of Jennings (1904) and Dellinger (1906) has shown their position to be untenable.

Instead of seeking in the movements of the Amoeba for a key to the structure of contractile protoplasm, the present study turns to the cilium. Here we find contractile tissue, microscopically speaking, in its simplest form. As the cilia are outgrowths of the cell protoplasm, there is every reason to suppose that exactly similar structures may exist within the cell body. Until we have applied to the protoplasm of the cell the methods best adapted to preserve and demonstrate the character of cilia, we are not justified in appealing to indefinite and undemonstrable fluids to do the work of solids.

The investigation falls naturally into two parts. The first is concerned with the best methods of preserving and demonstrating the structure of cilia. The second is the application of these methods to contractile protoplasm as represented in the Protozoa, smooth muscle and striped muscle.

I wish to acknowledge my indebtedness to Dr. C. F. Hodge, under whose direction the work has been done, for many helpful suggestions and criticisms; to Mr. L. N. Wilson, Librarian of the University, for aid in securing the literature, and to Dr. F. N. Duncan for permission to use part of his unpublished manuscript.

Historical: Literature on the Structure of Protoplasm. Sources.

Reference will be given to special papers at the end of this work. There have been a number of comprehensive treatises devoted to different phases of the subject that should, however, be mentioned at this time.

1. Haller, "Elementa Physiologiae," Vol. IV, P. 514, gives a summary of the old theories of muscular contraction.
2. Herman, in the "Handbuch der Physiologie," reviews the theories from Haller's time up to 1879.
3. Cornoy, "La Biologie Cellulaire," gives an excellent review of the conceptions of protoplasm between 1665 and 1865.
4. Bütschli, in his "Mikroskopische Schäume und das Protoplasma," gives an exhaustive review of the works on protoplasmic structure between 1860 and 1892.
5. The theories of the structure of protoplasm are briefly summed up by Delage, "L'Heredite," 1903, pp. 23-33.
6. Davis, "Studies on the plant cell," covers the literature on the botanical side.
7. Flemming, in Merkel and Bonnet's Ergebnisse, Vols. II, III, IV, V and VI, gives the recent literature on the cell. Few papers of any import are omitted from his exhaustive treatment.
8. The literature on the cilium is admirably brought together by Putter in Asher and Spiro's Ergebnisse, Vol. II, No. 2, pp. 1-102.
9. Fischer, "Fixirung, Farbung and Bau des Protoplasmas," presents a criticism of the methods of cytology.
10. Heidenhain in Asher and Spiro's Ergebnisse, Vols. VIII and X, gives a full review of the literature on the muscle.

Early Views.

Protoplasm Without Visible Organization and Contractile.[1]

The first to postulate an internal framework to explain the movements of protoplasm was Brücke (1861). Previous observers and

[1]The first conceptions of the nature of protoplasm were developed between 1665, the date of the discovery of the cell by Robert Hooke, and 1839, the date of Schwann's publication. We find it variously spoken of at this time as "matière ou substance organisatrice, matière formatrice, mati-

most of the investigators of his time conceived protoplasm to be semi-fluid, viscid substance without visible organization. Among those who did much to establish this view are to be mentioned Max Schultz (1855-1863), Haeckel (1862) and Kühne (1864). The first two worked with the protozoa, Schultz especially with the Rhizopods, Haeckel with the Radiolarians, Kühne upon protoplasm. All writers were, however, united in ascribing to protoplasm or "Sarcode" the property of contractility. Under these circumstances one is not surprised that they sought in this fundamental property the explanation of all protoplasmic movements. Although many investigators have opposed this view, it has had at all times a goodly number of supporters and at present seems to be gaining in favor. Besides Schultz, Haeckel and Kühne, referred to above, we should mention Reichert (1863), Brücke (1861), Cienkowsky (1863) and de Bary (1862 and 1864) as early holding this conception.

If protoplasmic movements were to be explained on the basis of contractility of protoplasm, it was necessary to assume some organization for this substance. Although Brücke did postulate a contractile

ère germinale," etc. (Cornoy, 1884, p. 176). In 1835, Dujardin designated it as "sarcode" in the infusoria. With Schleiden it was, "Schleim." (Beiträge zur Phytogenesis. Müller's Arch., 1838). The advances in microscopical anatomy during the years 1839 and 1840 gives Purkinje the credit of first using the term "protoplasm." Later, von Mohl ("On the Movements of Sap in the Interior of the Cell," Bot. Zeitung, 1846, p. 73) says, "The remainder of the cell is more or less densely filled with an opaque, viscid fluid of a white color, having granules intermingled in it, which fluid I call protoplasm."

The first to speak definitely of its properties was Dujardin. (Sur les Organismes inferieurs, Ann. Sc. Nat. 1835, p. 367.) In speaking of the sarcode he says, "Je propose de nommer ainsi ce que d'autres observateurs ont appéle une gelée vivant, cette matière glutineuse, diaphane, insoluble dans l'eau, se contractant en masses globuleuses. . . . enfin se trouvant dans tous les animaux inferieurs interposée aux autres élements de structure." Between 1840 and 1865 the work of Schultz, Haeckel, Williamson, and among the botanists, Naegeli, Cohn, de Bary, Cienkowsky, and many others did much to give us clear conceptions of protoplasm. Cornoy sums up the general notion of its properties at the end of this period in the following sentence: "Une masse diaphane, semi-liquide et visqueuse, extensible mais non élastique, homogène, c'est-a-dire sans structure, sans organisation visible. parsemée de nombreux granules et enfin essentiellement douée d'irritabilité et de contractilité."

framework, which was the beginning of our reticular theory of the structure of protoplasm, most of the investigators of his time were simply content to refer in a general way to contractility as an ultimate factor, not attempting further analysis.

Contractile Protoplasm Reticular or Fibrillar.

Although the early writers were united in ascribing to protoplasm the property of contractility, it was first with Brücke that this contractile substance took on organization. It is not very clear just what his views were, but it is evident he thought that protoplasm was made up of a firm, contractile reticulum bathed in a fluid. Two years later Cienkowsky assumed a similar structure for the plasmodia of the myxomycetes.

It was perfectly natural, therefore, for Heitzmann when he found a reticular framework in the protoplasm of Amoebae and the white corpuscles of a number of animals (Flusskeckers, Tritons and Menschen) to ascribe to this reticulum contractility, anl to see in it an explanation of all protoplasmic movements. (Heitzmann, 1873.) He also agreed with Brücke and Cienkowsky in that he thought this reticulum bathed by a non-contractile fluid. Bütschli (1892, p. 105) questions Heitzmann's observations, and thinks we should attach little value to them. However this may be, it is quite possible he came much nearer divining the true structural character of protoplasm than did Bütschli himself. A large number of investigators since Heitzmann's time have, in the main, agreed with him that protoplasm has a reticular or fibrillar structure and that it is the framework or fibrillae that is contractile, referring all phenomena of movement (locomotion, change of form, ciliary movement, etc.) to these structures.[2] Among those holding this point of view are to be

[2]We can trace back to an early date observations on the reticular or fibrillar structure of certain cells. Thus for the muscle, according to Heindenhain, we find Lauth (1834), Schwann (1839), Henle (1841) and Holst (1846), speaking of "feinere und parallele Fibrillen." Later the extensive works of Wagner (1863) and Rouget (1863) in the Gastropods, Nematodes, Lumbricus, and various higher animals, brought out beautiful fibrillae in the smooth muscles of these forms. Heidenhain has recently gone over the literature covering the muscle and I refer the reader to him for further history of this subject.

mentioned Schmitz (1880),[3] Reinke and Rodewald (1881-2), Cornoy

The fibrillar nature of certain nervous elements was first called to our attention by Remak (1837, 1843 and 1844). This doctrine was further developed by the works of Remak (1852), Stilling (1856), Leydig (1862 and 1864), Walter (1863), Deiters (1865), Arnold (1865 and 1866), Schultz (1868 and 1871), and Frommann (1867 and later), Bütschli.

Ciliated and non-ciliated epithelial cells were also shown by the work of Friedrich (1859), Eberth and Marchi (1866), Stuart (1867), Arnold (1875), Eimer (1877), and Nussbaum (1877) to contain fibrous or striated structures, Englemann (1880) for the ciliated epithelium and Leydig (1856), Henle (1866), Pflüger (1866, 1869, 1871) and Heidenhain (1868 and 1875) for the non-ciliated epithelial cells, describe similar structures. Frommann was first to advance the theory that some such structure is universally present in protoplasm, and, according to Bütschli, to him is due the credit of suggesting a possible reticular structure for protoplasm.

[3]Schmitz made his observations on plant tissues that were killed in a saturated solution of picric acid. He is convinced of the reticular nature of protoplasm and disputes the interpretation sometimes given that reticular structures are coagula.

In the years 1878 and 1879 Klein came forward in two publications as a supporter of the reticular structure of protoplasm. According to him cilia may be a continuation of fibrils within the cell.

Reinke and Rodewald and later Reinke and Katschmar, working with Aethalium septicum, expressed themselves as favoring the net-like structure of protoplasm. Their method was (somewhat) unique and deserves more attention than it has received. They subjected cakes of the plant to pressure and succeeded in pressing out about 66 per cent. of the fluid. From this they concluded that what remained behind was the substance of the framework. This framework is arranged in a spongy reticulum and is contractile. The fluid "Enchylema" fills the intertrabecular spaces and is kept from escaping from them by an enveloping layer of the same substance as the framework. Reinke's views are much more fully developed later (1899 and 1905). He argues very strongly against the conception of the active protoplasm being of a fluid nature and his comparison "fluid oars of a boat to flagellæ" is exactly to the point. Movements are always due, according to him, to the contraction of protoplasmic fibers.

The following quotation from Cornoy gives his point of view. "On peut admettre que le reticulum est seul doué d'irritabilité et de contractilité. C'est donc lui qui préside aux movements physiques, l'enchylema demeurant passif, ou a pu pres, dans cette categorie de phenomenes." It is thus seen that he thinks the protoplasm organized into a definite network, his reticulum, which is bathed in a fluid, the "*Enchylema*." Of these the reticulum alone is active. His reticulum corresponds to the "*mitom*" of Flemming and the "*protoplasma*" of Kupffer.

Rabl (1869) held the view that the systems of rays found in connection

with cell division are contractile fibrillae. These fibrillae arise from the reticulum of the protoplasm, during division, by the breaking down of the cross connections. After division they return to the reticular condition by anastomosing.

Van Beneden (1876 and 1883) held that the protoplasm contained a contractile reticulum and that it was a modification of this that formed the asters in ova.

Apathy (1891) holds to the fibrillar structure for nerves and muscles. He supports his views by later investigations.

Ballowitz, through numerous investigations between 1880 and 1890, gave very strong support to the fibrillar theory. Working with the spermatozoa he found evidence that convinced him of the fibrillar nature of contractile protoplasm. He believes that wherever we have contractility, fibrillae are to be found.

To go on through the list of contributions favoring this theory would take too much space. I will mention, however, some of the more recent papers bearing on this conception.

Schenck (1897 and 1900) came forward as an opponent to the fluid theory of protoplasm and as an advocate of fibrillar protoplasm. He thinks that Verworn's contractile fluids are not sufficient to explain protoplasmic movements and that contractile protoplasm (in the amoeba as well as elsewhere) "*muss eine bestimmte Structur haben, darf nicht als Flüssigkeit angesehen werden.*" And at another place "*die contractile Substanz fest ist.*"

The drawings of Arnold (1898) show beautiful fibrills in a large number of cells. He holds that this is the true structural character of protoplasm.

Allen (1903) working with the dividing pollen mother-cells of Larix holds that the fibers of the spindle arise from fibrills already present in the cell. That these fibers are contractile and that they control the activities of the cell. He argues against the view that they are precipitations as the result of killing.

Parker (1905) sees no reason why cilia might not be fibrillar and believes that their activities are best explained on the basis of such a structure.

Kunstler (1906) shows a beautiful reticular network in bacteria.

Hartmann (1906) thinks the active contractile part of protoplasm is fibrillar.

Duncan, working in this laboratory, has come to the conclusion (held by a number of writers) that the contractile elements in smooth muscle are fibrillar. Although he has not published his work the examination of a large number of forms has already been made and in every case he is able to demonstrate such structures.

In two investigations from this laboratory on the Amoeba, one published jointly with Dr. C. F. Hodge, the author is of the opinion that the movements of this form are only to be explained by the presence of a contractile framework and the last paper (Functions and Structures in A. proteus) collects the evidence for such structure.

(1884-5-6), Ballowitz (1884 and later, 1888 and 1890), Rabl (1889), Schneider (1891 and later), Klein (1878 and 1879), Apathy (1891), and, more recently, Heitzmann (1894), Schenk (1897 and 1900), Arnold (1898), Allen (1903), Schneider (1903 and 1905), Reinke (1905), Parker (1905), Dellinger (1906), Hodge and Dellinger (unpublished), etc.

Although in recent years the results of many investigations have been opposed to this theory of the structure of protoplasm, the last two researches just mentioned have taken away much of the foundation on which opposition was based, and there is little doubt that it will prove nearer correct than any developed to take its place.

Englemann's Inotagmas.

A modification of the fibrillar theory was brought forward by Englemann in 1868 and further developed by him in 1879 and '80. According to him, protoplasm is an aggregate of minute contractile and "reizbar Formelemente." The phenomena of movement are the result of the change of form of these minute elements. Englemann names these contractile elements "Inotagmen." He thinks of them as molecular in size, spherical in form when contracted, and thread-like when at rest. The reasons for these assumptions are: First, that protoplasm in however small masses takes on a spherical form when contracted. Second, that when relaxed protoplasm shows often fine fibrillar striations and is, in its finest division in contractile structures, a "*langgestreckte Form.*" Contraction is brought about by a change of turgidity, as the element would probably shorten with an increased turgidity and would stretch out again after giving off fluid.

Strasburger's Kinoplasm and Trophoplasm.

Strasburger (1892 and later) and many other botanists who have followed him divide protoplasm into two substances, his kinoplasm and trophoplasm. Of these the kinoplasm is active, entering into the formation of the fibrillae of the spindle and other active organs of the cell, such as the cilia, centrospheres, centrosomes and the cell membranes, while the trophoplasm is nutritive. The above classification implies a physiological difference in the two substances.

According to Davis (1904, p. 712), the kinoplasm is homogeneous in structure, either minutely granular or consisting of delicate fibrillae composed of very small granules placed end to end. Its homogeneous character is shown in the cell membranes, while the fibrous condition appears during cell activity and then disappears. On page 449 Davis says: "Kinoplasm runs through cycles in which the structure passes from a granular condition to a fibrillar and back again into a granular state." He admits that the fibrillae may not disappear, but simply be arranged in a closely packed network which is invisible under the microscope. All protoplasmic movements are due to the contraction of the kinoplasmic fibrillae. Although there is much about the kinoplasmic structure that is not understood, there is much in this theory of the structure of protoplasm that is attractive.

Hyaloplasm Active, Spongioplasm Inert.

One of the most peculiar theories developed to explain protoplasmic movements appeared in the year 1883. In this theory Brass (1883 and '84) and, soon after, Leydig (1885) exactly reverse the conception of the adherents of the reticular theory. Instead of the reticulum, the spongioplasm, according to these two writers, it is the fluid part, the hyaloplasm, that is contractile. In its activities is to be found an explanation of all protoplasmic movements. Few investigators have been inclined toward Leydig's hypothesis. Rhode (1890 and '91), Griesbach (1891) and Schäfer (1887, '91, '93 and 1904) about complete the list. Schäfer's views have been criticized by Bütschli (1892) and recently by Putter (1903), especially in its application to the cilium. Although Schäfer (1904) answers Putter's criticism, we find little in his discussion that warrants us in accepting his view. Recent investigations seem to prove conclusively that his position is untenable.

Schäfer's view may be summed up briefly as follows: Protoplasm is composed of two distinct substances, spongioplasm and hyaloplasm. "Spongioplasm has a reticular or sponge-like arrangement, an affinity for staining fluids, is firmer than hyaloplasm, but perhaps not actually solid, and is in all probability highly extensible and elastic. Hyaloplasm, on the other hand, is structureless, has little or no affinity for stains and is highly labile and fluent. It is by the active flowing of

the hyaloplasm, not by the contraction of the spongioplasm (as conceived by Cornoy), that the movements of cells are produced. Of the two substances, the hyaloplasm is the more active, the spongioplasm the more inert. The spongioplasm forms, in fact, a sort of framework supporting the hyaloplasm and into which, under the influence of stimuli, the hyaloplasm becomes wholly withdrawn" (Schäfer "On the Structure of Amoeboid Protoplasm," 1891, p. 195).

Objections to the Contraction Theory.

Observations, principally on the movements of the protoplasm in Amoebae and in plant cells, caused many observers to reject the contraction theory of the movements of protoplasm. In an advancing Amoeba the supposed currents which gave rise to the objections to contractility as a cause of protoplasmic movement were those beginning at the point of advance and extending backward. Wallich (1863) and Bütschli (1873) for Amoebae, and Hofmeister (1865 and '67) for the plant cell called attention to such currents. Since then many investigators have either thought they observed or else have assumed the presence of surface currents of this kind and have based their theories of protoplasmic movement on them.

Among those who took this standpoint were Hofmeister (1865 and 1867), Bütschli (1873), Quincke (1870 and later), Berthold (1872), and more recently Bütschli (1892), Rhumbler (1898, 1902, '03, '04 and '05), Gurwitsch (1904) and many others.

Unfortunately for the adherents of these theories, the recent investigations of Jennings (1904) and Dellinger (1906) have shown that the backward currents do not exist. It further appears that the real character of the movements in the Amoeba was not at all divined.

Protoplasm a Complex Fluid.

Berthold, Quincke, Schwartz, Rhumbler and others.

Although the doctrine of the fluid nature of protoplasm fell much into discredit during the seventies, it was taken up again in the eighties by a number of investigators.

Thus, Berthold, in his work published in 1886, came forward with arguments in favor of this conception which was universally

held earlier. He did not, however, try to support it by direct proof, but laid it down, rather, as a hypothesis upon which to base his observations and speculations upon the structure and movements of protoplasm. According to him, protoplasm is an emulsion; that is, it is a mixture of two or more complex fluids ("Der Plasmakörper in seiner Gesammtheit als eine Emulsion von mehr oder weniger flüssiger Consistenz aufzufassen ist." (Berthold 1886, p. 64.)

Quincke (1870 and later) also held that protoplasm was a fluid, but after Bütschli's publication seemed to favor the foam theory. It was from Quincke that Bütschli got the idea of foams which figure so prominently in his conceptions of protoplasm, but Quincke does not seem to have made the application of his foams to protoplasm until after Bütschli published.

Schwartz, another writer of this period (1897), seems to hold to the view of protoplasm as fluid, but, as Bütschli points out, it is difficult to understand just what his position is.

In recent years Verworn, Rhumbler, Jensen, Loeb, Gurwitch and many others have supported this view.

Bütschli's Schäume.

Very early Bütschli, as has been noted above, objected to the contraction theory of protoplasmic movements, and in 1887 we find him advocating the fluid nature of the endosarc of the Infusoria. Later (1892) he came out with a definite theory of the structure of protoplasm. According to him, the key to its structure is to be found in the microscopic oil-foams. The first fifty-seven pages are given up to the investigation of foams of different kinds. After an extensive study of the protoplasm of a large number of protozoa, of bacteria and of cells of many animal tissues, he concludes that its structure corresponds to that of the minutest microscopic foams. ("Nach meiner Auffassung entsprach der Aufbau des Plasmas den microskopischen Schaümen mit dem Unterschied, dass der Wabeninhalt gewöhnlicher Schäume hingegen eine wässerige Flüssigkeit sei." Bütschli, p. 3.) In the application of this conception to the explanation of protoplasmic movements he admits that, while it is satisfactory for amoeboid movement in the strict sense, modifications of it, especially

the formation of the fine pseudopods of many "Sarcodina," find no explanation. (Bütschli, p. 198.) He suggests (p. 208) that muscular contraction may possibly be explained by the above hypothesis. The fibrils are there composed of a row of alveoli instead of being simple threads.

Since Bütschli's publication many observers have described protoplasm as a "Schaum." Thus, Andrews (1897), after a most extensive microscopic study of living protoplasm, decides that all the phenomena observed finds an explanation in Bütschli's Schaumstructure. Crato, in 1892, '95 and '96, finds the foam structure to hold for plant protoplasm. Erlanger (1897) expresses himself in favor of this theory. Degen (1905), after a series of investigations on the action of the contractile vacuole, is of the opinion that protoplasm is alveolar. The alveoli of his photographs, it seems, however, might be interpreted as meshes of a reticulum.

Study of Cilia.

Point of View.

A number of eminent investigators, Bütschli, Rhumbler, Jensen and others, have taken Verworn's standpoint, that "Die lebendige Substanz der rhizopodoiden Zelle mit ihrer Bewegung muss Ausgangspunkt für die Untersuchungen der Contractionserscheinung sein. Es heisst die Lösung des Contractionsproblems unnöthig erschweren, wenn man die Behandlung bei der quergestreiften Muskelzelle beginnt, wo die Differenzirung der lebendigen Substanz und ihre einseitige Anpassung an eine bestimmte Leistung ihren höchsten Entwicklungsgrad und ihre grösste Complication erreicht hat," and have approached the problem from this point of view. Yet the outcome of their investigations in complex fluids and microscopic foams has been unsatisfactory and has led to widespread confusion of our notions of contractile structures. For pure contractile tissue, microscopically speaking, our simplest structure is the cilium. Here, with the protoplasm accessible for experiments with all kinds of reagents, if anywhere, we should find the key to the structure of contractile protoplasm. If, in applying the methods for the demonstration of cilia to the protoplasm of the cell, structural elements are demon-

strated, we may assume that these structures are real. It is from this point of view that the present investigation is pursued.

Finer Structure of Cilia.

"Ueber den feineren Bau aller genannten Organellen sind die Angaben überaus spärlich, die meisten Beobachter sahen nichts als hyalines Plasma" (Putter, 1903, p. 15). This is a fair statement of recent views. Although a study of ciliary action has led to certain fairly well-founded conclusions as to their probable structure, few direct observations of their finer anatomy have been made.

Englemann (1881, p. 541) points out that all large cilia, ciliary membranes, etc., are made up of fine fibrils. These fibrils correspond to those of all contractile structures.

Kliennenberg (1886) took the ground that the large "Geiseln der Wimperringe (bei Lopadorhynchus, Polycha) aus 20 bis 30 Cilien bestehen" (Putter).

Jensen (1887) figures, for the tail of sperms, fibrils that were brought out by pressure on the cover glass. Judging from his drawings the fibrillar structure is very evident.

Ballowitz (1886 and later), in a series of contributions to the structure of spermatozoa, gives some strong evidence for fibrillar structure in these organoids. In another paper (1890) he holds that contractile tissues, wherever found, are fibrillar. There is no doubt from his drawings that this is true, at least in the sperms.

Schuberg (1891) describes the "Zusammensetzung der Membranellen" of Stentor coeruleus and bursaria and of some hypotrichous infusorias. They consist, according to him, of two "Reihen" of fibrils.

Stevens (1901) asserts that the aboral membrane of Licnophora macfarlandii, Stevens, consists of fine long cilia, which are visible in the living animal under high magnification. (Putter.)

Theories of the Structure of Cilia.

Although there are a few direct observations on the finer anatomy of cilia, a number of theories of their probable structure have been advanced. These are admirably summed up by Putter, pages 27-29.

They are, in brief: First, that the cilia are lifeless processes attached to the cell. They are somewhat stiff and are moved by active

elements in the cell body. That is, they are "Bewegbar, nicht beweglich." This was the conception of the earliest observers and has been recently revived by Benda (1891). Although it has at all times had occasional supporters, Stuart[4] (1867), Nussbaum (1877), Claparedes (1875) and Kraft (1890), it has not, as a rule, appealed to investigators.

Second, that cilia are hollow elastic sheaths into which a fluid is injected and withdrawn. This is the hypothesis advanced by Schäfer in 1891 and supported by him recently against an attack by Putter. So far as I know, it has found practically no support among investigators, most of them agreeing with Parker that there is little in recent work to justify it. In fact, the continued activity of cilia after complete isolation indicates that they contain within themselves their own contractile material.

Third, that cilia are complex fluids. This is the view of Verworn (1890 and later), also of Jensen. This conception has been criticized, Schenke (1890), in which criticism he gives the reasons why contractile substance cannot be fluid. So far as cilia are concerned, Reinke possibly expressed the objections to this view best when he said: "Als flüssig oder halbflüssig können diese Geisseln unmöglich gelten. Sie wären dann so wenig imstande, die mechanische Arbeit der Fortbewegung der Spore zu leisten wie est möglich ist, ein Boot zu bewegen mit Rudern die aus einer Flüssigkeit bestehen."

Fourth, that cilia have a fibrillar structure and that the movements are due to the contraction of these fibrils. The fibrils may be only temporary arrangements of the molecules of an otherwise homogeneous substance. This is Putter's view. He thinks himself forced to it by lack of structure in the flagellum. I do not think his position is well taken, and if, as will be shown later in this paper, the flagella are really fibrillar, he has no ground for it whatever.

In Englemann's theory, first advocated by him in 1868 and devel-

[4]Stuart bases his view on observations made on ciliated cells of "Aolidenlarven." He saw running through the cells parallel fibers some of which went to the nucleus. During cell activity, he observed that when the cilia were in motion the nucleus was also affected, and concluded that the structures in question were contractile fibers by which the cilia and nucleus were moved.

oped further in 1879, the fibers are permanent structures. Cilia are, according to him, composed of fine elements, "Inotagmen," which in their resting condition are fibril like and when contracted are more or less rounded. They are arranged with their long axis parallel to that of the cilium. Parker (1905) has expressed himself as in favor of the fibrillar hypothesis, and thinks it "the most consistent thus far advanced as an explanation of the more usual types of ciliary movement." He does not agree in any way with Putter's objections, and suggests that even in the apparently homogeneous flagella there may be fibrillae.

Ballowitz (1886, '88 and '90), Jensen (1887) and others conceive the fibrillae as running the full length of the cilia. They have gathered much evidence which supports this view.

Effect of Killing Reagents on Cilia.

Introduction.

Although many writers have dealt with the effects of killing reagents on protoplasm, Berthold (1886), Fischer (1894 and 1899), Bütschli (1892), Hardy (1899), Tellyesniczky (1898), Wasitlewski (1899) and others, none so far as I have known have approached the question from the standpoint of visible structural elements of the living tissue. They have, rather, dealt with structures that appear after the application of the reagent. For that reason there has always been a question as to the reality of such elements. In selecting the cilium as a structure already present, which is as delicate as the finest structures demonstrated in the cell, it has been the thought of the writer that reagents which produce no change in it would likewise leave the elements in the cell undisturbed, and thus the structures found in the cell could be considered normal.

Methods and Materials.

Paramecium caudatum, Stylonychia pustulata and Actinosphaerium eichhornii were used. Probably all the cilia of Stylonychia are compound; that is, composed of a number of fibrils coiled together spirally (Pl. I, Fig. 1.) For this reason the effect of the reagents on its cilia are more easily observed, and it was used entirely for the

photographs. Actinosphaerium was chosen because of its long, finely drawn out pseudopods, which have a central core of fibrillae. In taking the records only the fibrillae were considered.

In all, twenty-eight different agents were tested. They represent the killing fluids generally used in cytological studies. For convenience I have arranged them in seven groups according to some one reagent they contain. Some reagents are found in more than one group.

All observations were made under a 1.5 mm. (Spencer Lens Co.) Objective and No. 1 eye-piece.

Osmic Acid Group.

This group contains osmic acid, 4 per cent., 1 per cent. and 2 per cent.; osmic acid 2 per cent., 95 per cent. alcohol, equal parts; 2 per cent. osmic acid mercuric bichloride saturated in normal salt, equal parts; 2 per cent. osmic acid, 10 per cent. formol, equal parts, and strong and weak Flemming. (If two or more reagents enter into a solution it was made up just before using. This holds for all reagents in all groups.) These reagents prove to be the more satisfactory in preserving cilia than those of any other group. In most cases the cilia are well fixed in nearly normal condition. Strong and weak Flemming are the exceptions.

Osmic acid:—Cilia treated with osmic acid in solutions of .4 per cent., 1 per cent. and 2 per cent. strength seem entirely normal. They are full length and straight. (Pl. I, Fig. 2). After making many tests with the three solutions the author favors the 2 per cent. solution as the most reliable.

Two per cent. osmic, 95 per cent. alcohol:—This combination is a fair killing agent.

Two per cent. osmic + mercuric bichloride:—This solution next to osmic acid gave the best results. Cilia treated with it are full length and straight.

Strong and weak Flemming:—These two solutions so universally used in cytological studies gave the most unsatisfactory results of any in this group. Cilia are apparently broken or twisted into what appears to be short, crinkled threads. (Pl. I, Fig. 3.) These threads

resemble the mitoms of Flemming, also those so often figured by botanists whenever a fibrillar structure is represented.

Two per cent. osmic + formol:—Cilia treated with this solution are straight and entire. In stylonychia the fibrils seem broken up, but the cilium, as a whole, is well preserved.

Mercuric Bichloride Group.

Saturated solution of $HgCl_2$ in normal salt, $HgCl_2$ and 2 per cent. osmic acid, equal parts Mann's Fluid, Rabl's Fluid, and HgCl + 95 per cent. alcohol. Mercuric bichloride gives better results when used alone than when used in any combination with other agents except osmic acid. In most cases the cilia are twisted, shrunken and broken. HgCl in combination with alcohol gave the poorest results. Fig. 4, Pl. I, shows a group of cilia killed with Rabl's Fluid. They are typical for the cilia killed with any reagent of this group.

Alcohol Group.

This group contains besides alcohol absolute, 95 per cent., and 50 per cent. alcohol in combination with $HgCl_2$, osmic acid and acetic acid. Alcohol with the exception of acetic acid gave the most unsatisfactory preparations of all the reagents tested. Cilia were often entirely destroyed. The animal was shrunken and in case of Stylonychia broken up. It was often difficult to find pieces for record. Pl. I, Fig. 5, shows one of the best groups of cilia found after treatment with absolute alcohol. Out of twenty animals only three or four showed any cilia.

Potassium Bichromate Group.

This agent enters into Müller's, Zenker's and Tellyesniczky's fluids. Cilia treated with these reagents are shortened. In Stylonychia the fibrills are much crinkled and twisted, in Actinosphaerium the fibrills are granulated. Zenker's fluid, which is used more than any other of the group, gives the best results, but with it the ends of the cilia are rounded and fused. (Pl. I, Fig. 7.)

Chromic and Acetic Acid.

These two agents enter into strong and weak Flemming and the common chromo-acetic acid solution. Chromic acid shrinks and fuses cilia. (Pl. I, Fig. 10.) Acetic acid melts them down into a mass of granules. (Pl. I, Figs. 8, 9.) The combination of the two fuse the cilia so that they appear matted. (Pl. I, Fig. 11.) The action of Flemming's solutions has been discussed above.

Picric Acid.

Picric acid enters into Mann's and Rabl's fluid, which I have placed in the HgCl group, and is used with acetic acid. Cilia treated with it are fairly well preserved. The ends are fused, but in some cases the fibrils remain quite distinct nearer the base. (Pl. I, Fig. 12.)

Formol.

Formol in 4 per cent. and 10 per cent. solutions and in a 1 per cent. solution of osmic acid in 5 per cent. formol was used. It is difficult to interpret the action of this agent. The body of the animal was poorly preserved. In the case of Stylonychia it was broken into fragments. The fibrils of the cilia attached to these fragments seemed well preserved, but the cilia themselves had their identity destroyed. The stronger solution gave the best results. (Pl. I, Fig. 13.)

Platinum Chloride.

A 1 per cent. solution of Platinum chloride was tested. It yielded no results to recommend it.

Conclusions.

Cilia treated with different reagents show a marked difference in structure. Of the twenty-eight agents tested osmic acid (.4 per cent. to 2 per cent.) is the only one that preserves the cilia in normal condition. Wherever it enters into a killing fluid its influence can be seen. All other reagents produce marked changes in cilia which are characteristic for each reagent.

In interpreting the structures revealed in tissues killed with these reagents their action on fibrillar structures should be taken into

account. We should not expect the fibrillar structure to remain normal in tissue treated with most of them. Fibrillae in the cell should show the same structure that cilia show after treatment with any one reagent. Thus, osmic acid would leave the fibrillae normal, strong and weak Flemming would break them up into short twisted threads, mercuric bichloride would in some cases leave them almost normal (HgCl sat. in normal salt), and in others fuse them so they entirely disappear. Alcohol would break them up in densely granulated masses, acetic acid would leave them more granular than alcohol, chromo-acetic and picric acid would leave them fused masses, while formol would show them as threads.

Structure of Flagella as Demonstrated by Teasing.

Introduction.

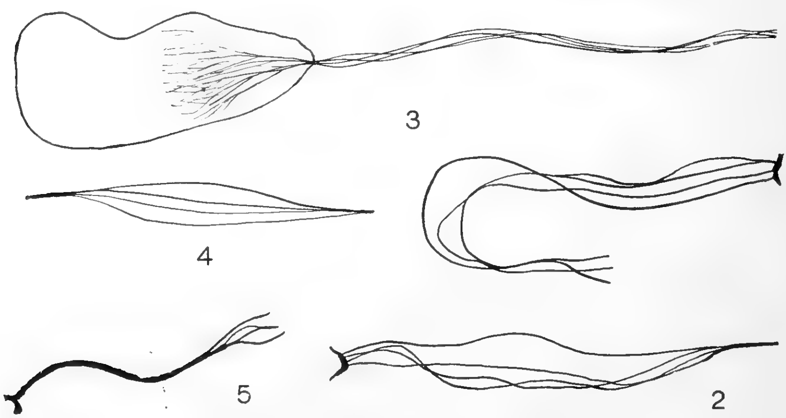
There is almost universal agreement in the literature on the Flagellum that this organ is "Ein homogener dünner Faden, der keinerlei besondere Anhängsel trägt." This is the view of Kunstler, Löffler, Bütschli, Klebs, Hertwig and, more recently, Putter. In 1894 Fischer came forward with the view that a number of flagella, the Flimmergeissel (Euglena viridis and Monas guttula), "Besteht aus einem homogenen Faden, der mit einer oder mehreren Reihen kurzer, dünner, zugespitzter Härchen (Cilien) besetzt ist." On the other hand, another class of Flagella, the "Peitschengeissel," consists of a homogeneous thread that often shows fibrillae clinging to it. So far as I know, Fischer's results have not been confirmed; and my own observations have yielded nothing approaching his figures or descriptions. Putter, because of the structure of the flagellum, rejected the fibrillar theory of the structure of cilia. I hope to show that in its structure is really the best justification for his theory.

Method and Materials.

For this investigation the flagella of Euglena, Chilomonas paramecium and Spirillum (Sp. ?) were used. Mounts from infusions containing the first two of these forms were placed under a 1.5 mm. oil immersion and subjected to pressure.

Euglena.

The flagellum of Euglena is composed of four fibrils which extend its entire length. They are twisted about one another in a spiral of two and one-half turns. (Figs. 1, 2 and 3.) This structure (Pl. II, Figs. 1 and 2) is demonstrated with ease by subjecting a flagellum to slight pressure. The fibers gradually untwist or separate so that each is distinctly seen. They can be traced into the animal, where they branch out into a system of rootlets. (Fig. 3.) These fibers probably explain Fischer's thread-like appendages to his "Peitschen-



FIGS. 1 and 2.—The uncoiled fibrils of the flagella of Euglena.
FIG. 3.—Euglena with the fibrils of the flagellum branching out into a system of rootlets in the protoplasm of the body.
FIGS. 4 and 5.—Flagella of Chilomonas paramecium.

geissel." What he really saw was some of the uncoiled fibers. Although I have used his methods, I have been unable to demonstrate any other structure. I am inclined to think that the fine cilia along the flagellum of Euglena veridis, which he describes, were artifacts. Possibly he may have worked with different species.

Chilomonas Paramecium.

This form has two flagella, which are much smaller than the flagellum of Euglena. The structure was therefore more difficult to make out. Under the same treatment, however, they were demon-

strated to consist of four fibrills which had a spiral arrangement. (Fig. 4 and 5.) They were not traced into the cell and their relation to the cell body was not determined.

Spirillum.

With flagella of bacteria we descend the scale to still more minute forms which are not visible by ordinary histological methods. Still, no question can exist as to their active contractions. Thus, in any tissue cell there may be contractile elements or fibrillae which are invisible by ordinary methods. If simple, single-fibril, cilia or flagella exist, we should expect to find them among these most minute organs of bacteria. It is proposed to devote a special research to this point. At present I am able to offer the evidence from a single type. A large Spirillum common in stagnant water when stained by Loeffler's method shows unmistakably that its long, apparently simple flagellum consists again of four spirally-wound fibrillae. While not demonstrated alive by the teasing method used for Euglena and chilomonas, it is not difficult to find among the stained specimens a complete series from the apparently solid, simple flagellum through all stages of uncoiling to four distinct fibrillae. (Pl. IV, Fig. 8 and Text Fig. 5a.)

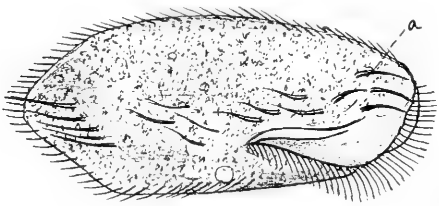
Theoretical Considerations.

As mentioned above, Putter (1903), after a discussion of the fibrillar theory of the structure of cilia, rejected it because of the difficulties met with in applying it to the movements of the flagellum. Parker (1905), in a discussion of the probable structure of cilia, does not agree with Putter and prophesies that even flagella may be fibrillar. From the above observations it is evident that this is true, at least, for three flagella. In these observations Putter's objections to the fibrillar theory become groundless, and the scant attention paid Schäfer's tube theory of ciliary structure is also justified.

Although only three have been demonstrated to be fibrillar, these make it probable that all flagella have a similar structure. The work on cilia to follow proves that they are constructed on the same general plan, though different cilia vary in the number of their component fibrillae.

Structure of Cilia Demonstrated by Teasing.

It has long been known that the large cilia of the Hypotrichia (Fig. 6) are composed of many fine fibrils twisted together. In a



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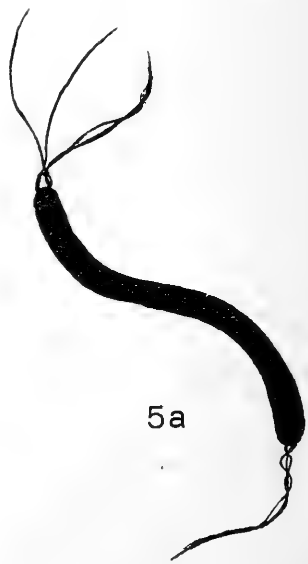

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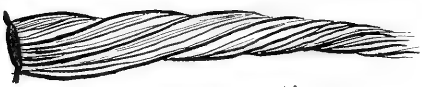

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Fig. 5a.—Spirillum drawn from photograph of Plate IV, Fig. 8.
Fig. 6.—Stylonychia showing types of cilia. (From Conn.).
Fig. 7.—A of Fig. 6 much enlarged.
Fig. 8.—Pseudopod of Actinosphaerium Eich. showing axil filament. (From Calkins' Protozoa, p. 80).

study of these forms the author became convinced that all their cilia had a similar structure. Using the same methods which brought out the structure of flagella, the fibrillar nature of these cilia was easily demonstrated.

The structure of the large cilia (Fig. 7) is that shown in Fig. 3, Pl. II. It is seen that they are composed of a large number of fine fibrils. In life these fibrils are wound together so that the whole appears perfectly homogeneous. In animals subjected to pressure or killed in 2 per cent. osmic acid the separate fibrils appear. In an animal that is breaking to pieces under pressure their fibrillar nature is best seen. (Fig. 6.) All the cilia of the Hypotrichs are made up in a similar way. They are composed of several fibrils which separate under pressure. (Pl. II, Fig. 4.) These cilia vary greatly in size and in the number of fibrils they contain. In many of them it is impossible to distinguish the fibrils that formed each individual after they have become separated. It is only on account of the size and arrangement of the cilia that it is possible to be sure the fibrils are not separate cilia.

The bearing of these observations on the theories of ciliary structure is evident. If in the Hypotrichia we have a series of cilia growing smaller and smaller, all composed of definite fibrils which can be separated, where are we to assume that this fibrillar structure ceases and cilia become homogeneous fibers or tubes of fluid? At the place where we are unable to demonstrate a fibrillar structure? This does not seem reasonable, for our means of demonstrating these structures are by far too gross. The simplest theory is that fibrillar structure extends on down the scale, although we are unable to demonstrate it. What we know of flagella makes this probable.

Application of Methods to Other Contractile Tissues.

Amoeba.

The Amoeba has been used by many recent investigators, Bütschli, Berthold, Quincke, Verworn, Rhumbler and others, as the starting point for their studies on contractile protoplasm. Unfortunately, in their investigations they did not apply modern histological technique to demonstrate structure in this form, hence the outcome of their work in microscopical foams, colloidal fluids, etc., as an explanation of amoeboid movement has resulted in widespread confusion in all our theories of protoplasmic structure.

Application of the methods and reagents which give the best prepa-

rations of cilia show in the Amoeba a definite framework of tissue. This framework is a finely meshed structure, essentially the same for the "endosarc" and "ectosarc." It is so woven as to form trabeculae with large inter-trabecular spaces in the interior, and, without essential differentiation, forms the outer (Pl. II, Figs. 5 and 6) membrane, the wall of the contractile vacuole, the walls of all food vacuoles, and the nuclear membrane. This structure is constant in all these relations and cannot be considered an artifact.

In a recent paper, "Locomotion of Amoeba and Allied Forms," the author has shown that the movements of Amoebae in no way correspond to the movements of complex fluids. On the contrary, it is pointed out that Hertwig's contention, that "Das Protoplasma ist kein Gemengsel zweier nicht mischbarer Flüssigkeiten, wie Wasser und Oel, sondern besteht aus einer Verbindung fester, organischer Substanzteilchen mit reichlichem Wasser," holds at every point. In this paper it is suggested that a reticulum of contractile tissue would explain all the facts of amoeboid movement. Since an application of the methods best adapted to preserve cilia, which we know to be contractile tissue, demonstrates a reticulum in Amoebae, and since the presence of a contractile reticulum is the simplest explanation of all the facts of amoeboid movement, I think we are justified in assuming *that the reticulum demonstrated is contractile.*

In a paper, "Functions and Structures in Amoeba Proteus," Hodge and Dellinger, which is soon to appear from this laboratory, a full report of the work on Amoeba is given, hence this brief reference to the part that bears upon my subject:

Actinosphaerium.

The literature on the structure of the Heliozoa is extremely limited. Bütschli (1892) speaks briefly of the protoplasm of Actinosphaerium and Actinophyrs. According to him, their protoplasm shows a finely-meshed structure in the ectoplasm, endoplasm and the pseudopods. This structure is not altered in killing with Flemming's fluid, from which he concludes that it is normal. In speaking of the ectoplasm, he says: "After treatment with the osmic mixture already mentioned the meshed structure is everywhere easily recognizable in the ecto-

sarc. Whether or not the ultimate structure of the axil thread is similar was a point not successfully determined, though it occasionally appeared to be so." The axil thread is the apparently solid core of the pseudopod. According to Calkins (Protozoa, p. 81), this thread is composed of "some unknown substance," which is "Probably stiffened protoplasm similar to the central axis of the reticulate pseudopodia." Fig. 8, taken from Calkin's "Protozoa," shows this axil filament and the surrounding protoplasm according to his view. My own work shows that the filament is really a bundle of fibrils which are probably contractile.

Structure.

In this investigation Actinosphaerium eichornii was used. Animals killed in 2 per cent. osmic acid and studied in dilute glycerine show a definite reticular network which forms the substance of the trabeculæ that surround the large inter-trabecular spaces. (Pl. II, Fig. 7.) The fibers of the trabeculae often unite to form larger fibers, which in a few cases were traced to the axil filament of the pseudopodia.

That we are dealing with a reticulum and not alveoli, as held by Bütschli, is evident from the following observations. The fibrillae of the pseudopods could in a few cases be traced into the reticulum and evidently furnished some of its fibers. (Pl. II, Fig. 10). Fibrillae could be found that were branching into smaller fibrils, and in these cases alveoli were often found in the angles. (Pl. II, Fig. 9; Pl. III, Fig. 1.) Sometimes the angle was completely rounded out by the alveolus. (Pl. II, Fig. 9, and Pl. III, Fig. 4.)

In the course of this investigation an explanation was suggested to me by Dr. Hodge which accounts for all the observations of alveolar protoplasm. It was this: If a viscid fluid bathing a reticulum would tend to form alveoli in the meshes of the reticulum and round out their angles, a perfect alveolar appearance would obtain. That such alveoli do tend to form on the angles is seen in Fig. 1, Pl. III.

Acting on this suggestion, experiments were made to determine to what extent this would hold in oil foams. Silk thread was teased into its ultimate fibers in an oil foam and mounted under a cover

glass. Pl. III, Fig. 2, shows the form the alveoli took. They are seen to be in the angles of the crossed threads, and their general arrangement is governed by the fibers. Another preparation was made in which no threads were placed. Pl. III, Fig. 3, shows the form of the alveoli in this case. Pl. III, Fig. 4, is the protoplasm of Actinosphaerium. It is evident that Figs. 2 and 4 are structurally alike, and one is convinced that there is fibrillar structure present in the protoplasm of Actinosphaerium, which controls the arrangement of the alveoli. The arrangement of alveoli in definite lines was often remarked upon by Bütschli. This definite arrangement finds an explanation in the above observations. Protoplasm, then, may be a mixture of fibrillae with foams, the arrangement of the alveoli being governed by the fibrillae.

So far as I know, there is nothing in this hypothesis that is contradictory to what we know. On the other hand, it would explain the presence of alveoli where a reticulum is demanded. It remains for investigation to show how far this explanation holds good. The above observations tend to strongly support it. In a later section other evidence will be adduced.

Probably more interest attaches to the work on the pseudopods than to that on the body of the animal. As mentioned above, the pseudopod has an axil filament that has generally been supposed to be simply a supporting mechanism. This filament runs through the ectosarc and ends near a nucleus in the endosarc. It is usually figured as if it were perfectly homogeneous (Fig. 8), but according to this conception it is difficult to see how it is formed and how, under stimuli, it is drawn into the body.

While studying the pseudopods of Actinosphaerium to determine the effects of different reagents it was noticed that the axil filament was almost always more or less fibrillar. Following out this suggestion, I made a careful study of pseudopodia killed in 2 per cent. osmic acid. By teasing such specimens, the fibrillar nature of the axil thread becomes quite distinct. (Pl. III, Figs. 5, 6, 7, 8.) Although it was difficult to trace the relations of these fibrillae inside the body, examples were found which seemed to indicate that they. in part at least, branch out into the meshwork of the trabeculae.

(Pl. II, Fig. 10.) These observations are in agreement with those of Bütschli on the pseudopodia of Actinophyrs. He says: "The protoplasm of the pseudopodia appeared in part composed of distinct longitudinal fibers. Moreover, this fibrillar modification of the protoplasm could be followed through the coarsely vesicular ectoplasm into the finely meshed endoplasm, and at the same time it could be demonstrated that the fibrous tracts pass into the meshwork of the endosarc."

Whether or not the fibrillae of the pseudopodia and of the body reticulum are contractile was not determined with certainty. However, one set of observations indicates that it is. Throughout the entire series of studies Ehrlich's blood stain in dilute glycerine (one drop of stain to one cc. of 10 per cent. glycerine) was used to stain the tissues. It was found that this stain picked out the contractile stalk of Vorticella, the ciliary bands of Vorticella and Stentor with their cilia, and the fibrillae of smooth and striped muscle. It is admitted that these are the contractile elements in these forms. This same stain picked out the fibrils of the pseudopodia of Actinosphaerium and the reticulum of the body, which indicates that we have here, also, contractile tissue.

Stentor.

The contractile elements of Stentor have been described by a number of writers (Metschnikoff, Johnson and Bütschli). They are easily recognized as the cilia and myonemes in the living animal, but require special methods to demonstrate their nature.

The cilia are of two kinds, those surrounding the peristome, and the body cilia. The former are complex; that is, composed of a large number of delicate fibrils. Pl. IV, Figs. 1 and 2, show the cilia after treatment with osmic acid and strong Flemming. From Pl. IV, Fig. 2, and Text Fig. 9 it is seen that they are extended into the body as an apparently solid mass. The relations or meaning of this part of the cilia were not determined. It is much like the solid ends of the fibrous core of the pseudopods of Actinosphaerium. The finer structure of the body cilia could not be demonstrated, but in many cases of partly melted down cilia they showed every indication

of being composed of fibrils. However, I am not ready to say that this is the case. It remains to be seen what a more powerful magnification will show.

The myonemes in specimens partially teased and killed in 2 per cent. osmic acid show a definite fibrillar structure after staining with the Ehrlich-glycerine mixture. (Pl. IV, Figs. 3 and 4.) Pl. IV, Fig. 3, which is a small section of a myoneme teased away from the body, indicates that they send branches into the body. Other observations led me to think that is the case. The body cilia are all outgrowths from myonemes. Stentor thus supports the theory that contractile tissue is fibrillar.

Epistylis.

This form was killed and stained by the same methods as given above. The stain quickly picks out the contractile elements. These are the cilia, the ciliary bands around the peristome and the myonemes which extend from the outer peristomal band to the point of attachment at the stalk. (Fig. 10.) The definiteness with which the stain picks out these structures leaves no doubt as to their identity. The presence of the contractile elements in these positions explain all the movements of the animal.

No data was obtained as to the finer structure of the cilia or the peristomal bands. In a teased specimen the finer structure of the myonemes was demonstrated. They are distinctly fibrillar, as shown in Fig. 11, probably send fibrillae into the body. Their relation to the outer peristomal band is also shown in the above figure. In epistylis the myonemes end at the junction of the body with the non-contractile stalk.

Smooth Muscle.

The major part of the work on smooth muscle was done by Duncan in this laboratory at the same time I was working with the contractile tissue in other forms. Reference will be made to his paper later. He did not investigate the stalk of vorticella, which is generally recognized as the starting point for investigations on smooth muscle. Although there are two well-established views as to the seat of contractility in the stalks of vorticella, I think there is no doubt that

the muscle-like spasmoneme is the contractile element. It is picked out immediately by the above Ehrlich-glycerine mixture and, with the myonemes and the cilia, is stained bright red when there is no stain in the rest of the animal.

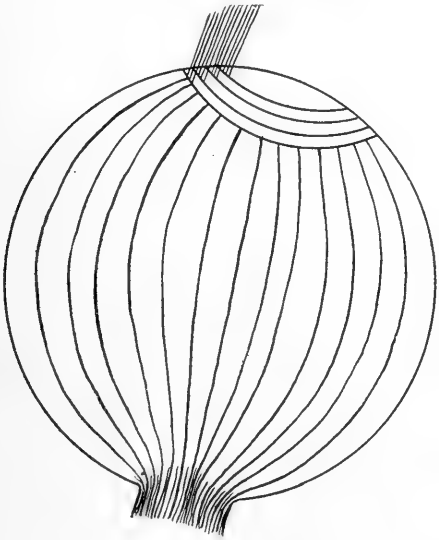

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Fig. 9.—Oral cilia of Stentor killed in strong Flemming.
Fig. 10.—Contractile elements of Epistylis.
Fig. 11.—Teased Myonome of Epistylis.

No structure could be demonstrated in the spasmoneme itself. Pl. IV, Fig. 6, is a microphotograph taken with an Ultraviolet Microscope. In this photograph the spasmoneme appears perfectly homogeneous; on the other hand, its relation to the myonemes indicates

that it is fibrillar. It is in reality the continuation of these myonemes into the stalk, and we should expect them to retain their structure, as they retain their function in this position. (Fig. 12.) Although no structure has been demonstrated, I do not think we are justified in assuming that it is homogeneous. It is probable that when a method is found to demonstrate it the structure will be found to be fibrillar.

As mentioned above, the major part of the work on smooth muscle was done by Dr. Duncan in this laboratory, and his results are ready

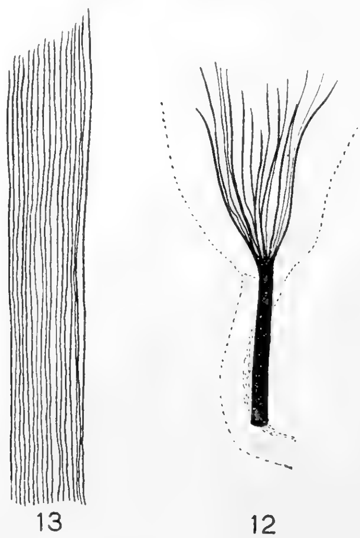

Fig. 12.—Myonomes of Carcesium.
Fig. 13.—Smooth muscle fiber taken from earthworm. (After Duncan.)

for publication. He used the same stain and, in many cases, the same killing reagents that I have used. His results are in agreement with what I have found in my studies. He finds that the smooth muscle in all the places he examined it (Hydra, sea anemone, star fish clam, Tubifex, Lumbricus, and cat) is distinctly fibrillar. Fig. 13, copied from his drawing of the muscle of an earthworm, shows the fibrillar structure in a portion of one of its fibers. The following quotation gives his general conclusions: "All of the structures that we have observed in contractile tissue force upon one the conviction

that it is composed of fibrillae and interfibrillar substance. However they may have arisen, they are a fact in the structure and not an 'Accident of structure,' as Bütschli maintains. In every case in which I have studied contractile tissue it has been composed of fibrillae or a single fibril running out from a cell."

Discussion of Results.

Present authorities are perhaps about equally divided on the question of the fibrillar, alveolar or colloidal-fluid nature of contractile protoplasm. In the cilium we have this most interesting substance in not only its purest, but also its simplest form. It is thus the natural point from which to begin the study of its structure; and by taking advantage of the natural path afforded by a comparative series of typical cilia, it seems possible to gain clearer conceptions than those attained through any other line of approach. The general conclusion is that contractile protoplasm is fibrillar in all the forms studied.

The fibrillar structure is preserved in its normal appearance by but few of the ordinary killing reagents. The great majority so alter the fibrillae that they are no longer recognizable. This fact goes far toward explaining the confusion in current ideas of protoplasmic structure. Before we deny fibrillar structure in any tissue we clearly must determine whether the reagent used preserves fibrillae. The beginning which has been made in my investigation of the effects of killing reagents on cilia leads us to conclude that osmic acid alone can be depended upon to preserve fibrillae in their normal appearance. Anyone who will watch cilia dissolve or change into granules during a second's contact with an unfavorable reagent will require no further argument on this point. Naturally, some fibrillae are more resistant than others. Thus, the fibrillae in the pseudopods of Actinosphaerium were left intact by reagents which completely destroy their identity in the cilia of Stylonychia.

A point of special importance is the result gained by teasing flagella fresh under high powers of the microscope. Structures revealed by this method are in no wise open to the criticism that they are artifacts produced by killing reagents. The ease with which the flagella studied are resolved into their component fibrils and the clear-

ness with which the method demonstrates the fibrillae of the axil filament in the pseudopod of Actinosphaerium leads one to think that this line of approach, or some modification of it, might yield valuable evidence as to the structure of the neurite and axis cylinder of nerve fibers. My observations on flagella and cilia as they pass under different reagents from fibrillar to meshed or granular debris leads us to conclusions as to the action of killing reagents on protoplasm different from those arrived at by Fisher and Hardy. Instead of considering all fibrillar structures as artifacts, it would seem that few investigators have divined the true fibrillar nature of protoplasm, because they have used reagents that destroy or modify the fibrils.

Alveoli exist in protoplasm, but reagents which preserve cilia never yield completely alveolar structures that could possibly be interpreted as Bütschli's "Schäume." The comparison of the alveolar protoplasm of Actinosphaerium, or of any of Bütschli's Schäume, as he figures them, with simple emulsions and with emulsions permeated with delicate fibers, leaves little doubt that the alveoli of protoplasm have their arrangement determined by fibrillae. This compromise interpretation uniting the fibrillar and alveolar theories of the structure of protoplasm has been offered by other writers, and is rather a matter for further investigation than for discussion in this paper.

The resolution of the flagellum of Euglena, Chilomonas and spirillum into four distinct fibrils and the demonstration of the fibrillar structure of the cilia of Stylonychia leaves the way clear for acceptance of not only the fibrillar theory of the structure of cilia, but also for the conception of fibrillae as a component of any protoplasm. With its acceptance, all theories which regard cilia as tubes or complex fluids appear gratuitous complications, and hence untenable.

In cilia or flagella capable of movement in all directions there should be at least four contractile fibrils. I have succeeded in demonstrating these in a typical series of forms. The presence of these four fibrils not fused or cemented together, but coiled in a long spiral, accounts for all their complex movements. Parker ('05) has pointed out that in cilia incapable of reversing only one contractile filament is necessary, if this is attached to an elastic supporting rod. With

reversible cilia both fibrils must possess the power of contracting, each alternately or under different stimuli, acting as elastic support for the other.

As I have watched and studied cilia and other contractile fibrillae for the past four years, comparing at every step the living with the fixed structure, I have observed many things that cannot be detailed in this paper, but I have seen nothing which contradicts the fibrillar theory of contractile protoplasm. No one who has watched the basal cilia of Vorticella protrude and be absorbed, often within a few seconds, can deny great plasticity to the contractile fibril; but, while it contracts and lashes the water or pulls hard enough to pinch a paramoecium in two, formed it must be, and to think of it as a strand of fluid baffles imagination. No one who has watched an amoeba divide can deny to the fibrils the power of auto-section or amputation, probably the power to liquefy at certain points; nor can one who has watched a Vorticella attach itself and grow its stalk question that the contractile fibrils have the power to cement themselves to foreign substances, and this, it would seem, must carry the conclusion that they fuse with one another. On the other hand, those who hold that contractile protoplasm is a complex fluid and that all fibrillar structure demonstrated in it is the result of fixing reagents, must explain why a reagent which preserves fibrillae outside the cell might not preserve them within the cell, and also why a reagent that destroys the fibrillae outside might not be expected to change those inside the cell. They must also bring forward a satisfactory explanation for the fibrillae which can be so clearly demonstrated in cilia and flagella of the living cell.

Conclusions.

1. Osmic acid is a satisfactory fixing reagent for the contractile structures investigated.

2. To interpret structures inside a cell after fixing with any reagent we must take into account alterations produced by it in fibrillar structures outside the cell.

3. Absence of fibrillar structure may mean that the fixing reagent used has destroyed the fibrillae.

4. The cilia of Stylonychia are composed of spirally coiled fibrils;

and the flagella of Euglena, Chilomonas and Spirillum are composed of four spiral filaments.

5. The axil filament in the pseudopod of Actinosphaerium is fibrillar.

6. The protoplasm of Actinosphaerium is reticular as well as alveolar. The definite arrangement of alveoli in any protoplasm is probably due to the presence of fibrils.

7. The contractile elements of tissues investigated are in every case fibrillar or reticular.

Clark University, May 31, 1907.

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DESCRIPTION OF PLATES.

Plates I, II, III, and IV are photographs taken with Spencer Lens 1.5 mm. objective and No. 3 eyepiece.

PLATE I.

Fig. 1 to 13 Cilia of Stylonychia.

Fig. 1 and 2.—Cilia killed with 2 per cent. cosmic acid.

Fig. 3.—Cilia killed with Strong Flemming.

Fig. 4.—Cilia killed with Rabl's fluid.

Fig. 5.—Cilia killed with Absolute alcohol.

Fig. 6.—Cilia killed with Tellyesniczky's fluid.

Fig. 7.—Cilia killed with Zenker's fluid.

Figs. 8 and 9.—Cilia killed with Acetic acid.

Fig. 10.—Cilia killed with Chromic acid.

Fig. 11.—Cilia killed with Chromo-acetic acid.

Fig. 12.—Cilia killed with Picric acid.

Fig. 13.—Cilia killed with Formol.

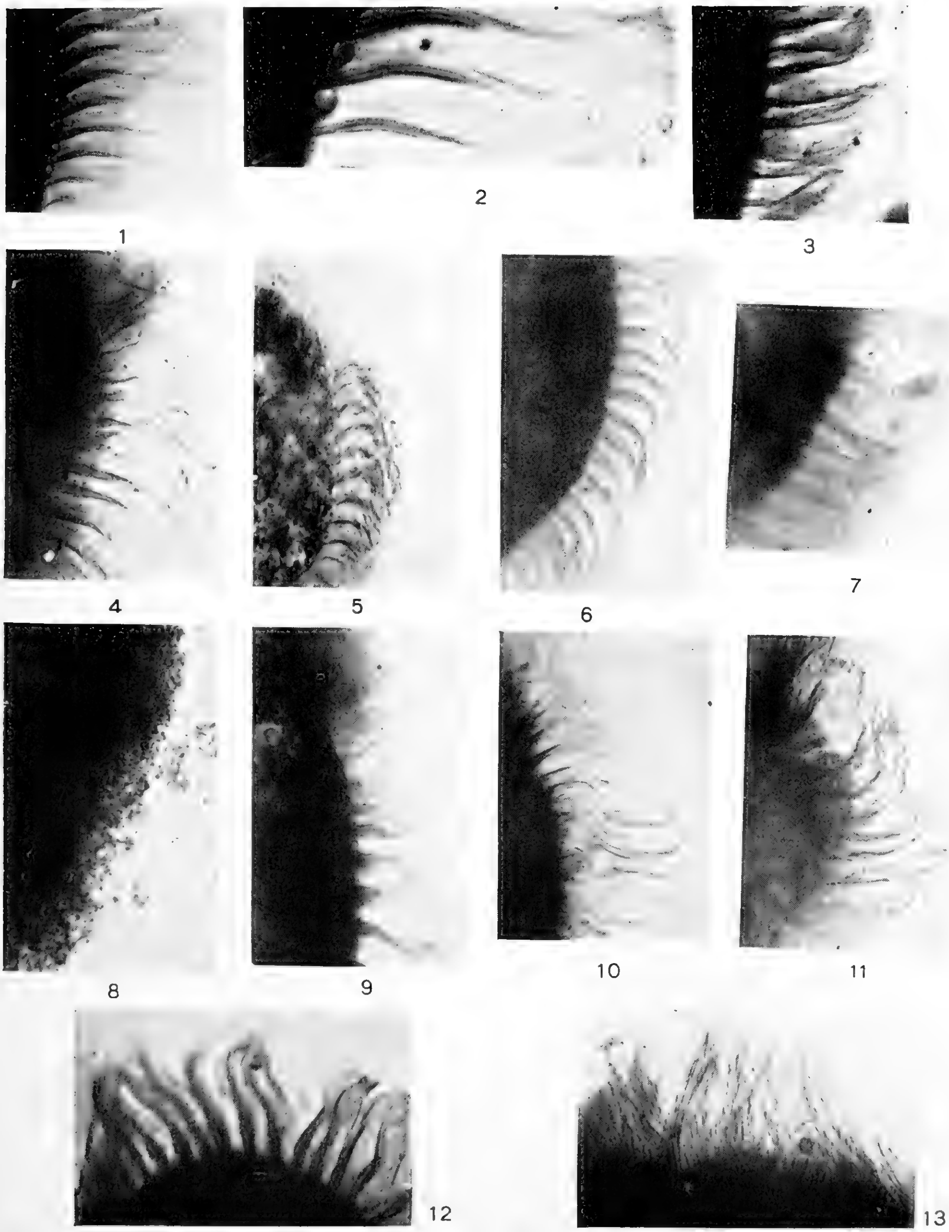
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PLATE II.

Fig. 1.—Flagellum of Euglena.

Figs. 1a and 2.—Flagella of Euglena which have been teased.

Fig. 3.—Large cilia of Stylonychia.

Fig. 4.—Ordinary cilia of Stylonychia.

Fig. 5.—Section of Amoeba showing the reticulum of the trabeculae. Killed in 2 per cent. osmic acid. Stained in gentian violet.

Fig. 6.—Portion of amoeba killed in 2 per cent. osmic acid and stained in gentian violet.

Fig. 7.—Small part of a section of Actinosphaerium showing the reticulum of the trabeculae.

Fig. 8.—Trabeculae of the reticulum of the "ectosarc" of Actinosphaerium.

Fig. 9.—Alveoli in the angles of branched fibrillae. From Actinosphaerium.

Fig. 10.—Fibrillae of the pseudopods of Actinosphaerium branching into the reticulum of the body.

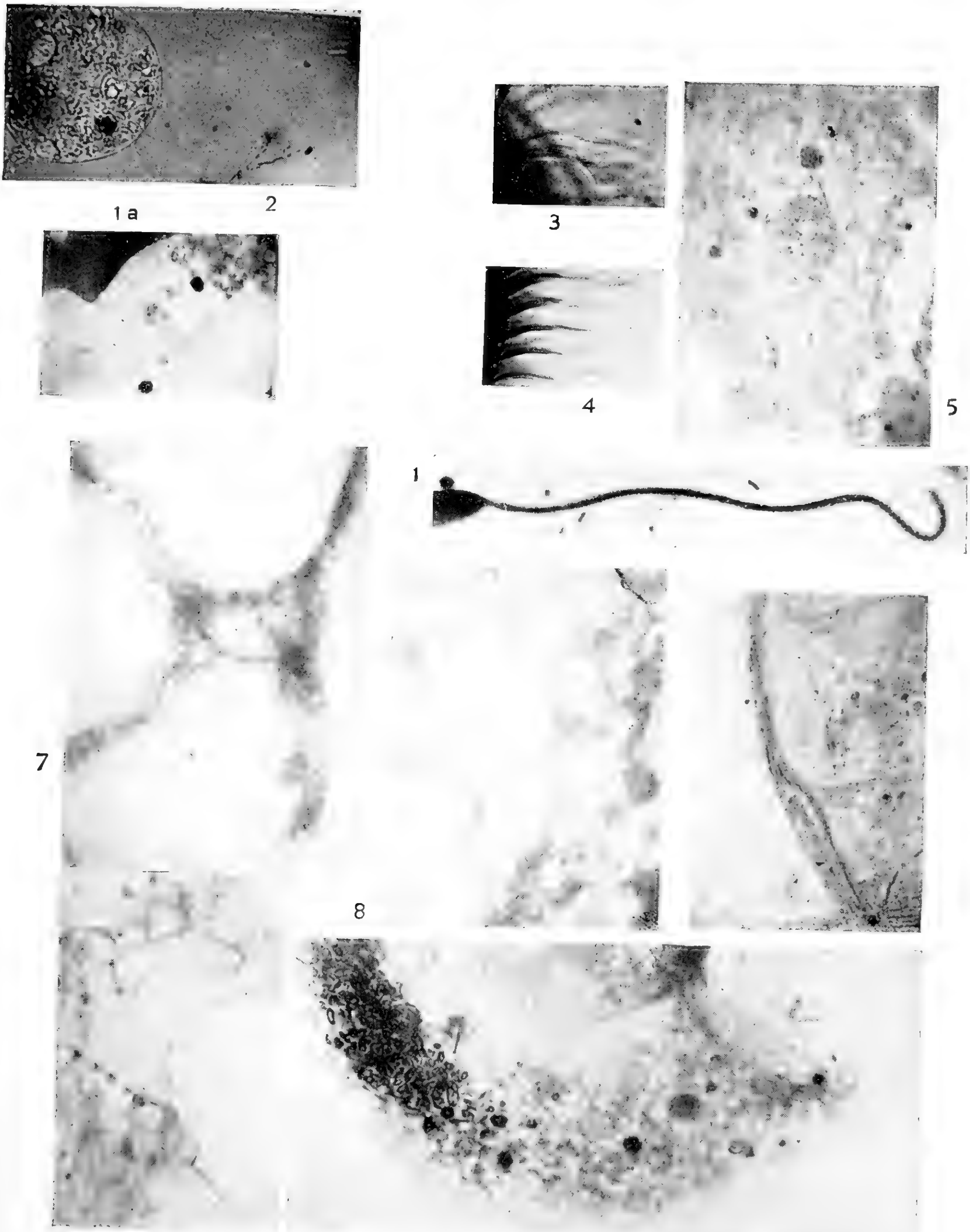
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PLATE III.

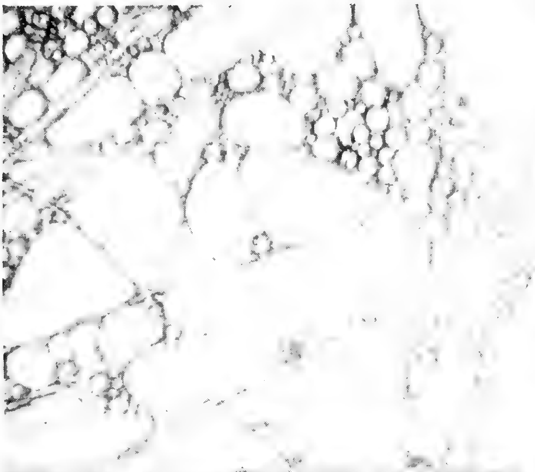
Fig. 1.—Fibril teased from the protoplasm of Actinosphaerium showing the alveoli at the angles.

Fig. 2.—An emulsion permeated with fibrils showing how the form and arrangement of the alveoli is conditioned by the presence of the fibers.

Fig. 3.—A part of the above emulsion without fibers.

Fig. 4.—Protoplasm of Actinosphaerium.

Figs. 5, 6, 7, and 8.—Fibrillae of the pseudopods of Actinosphaerium.



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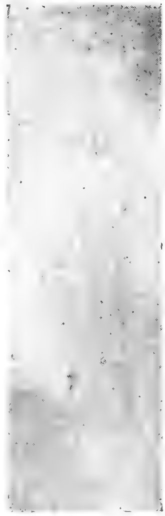

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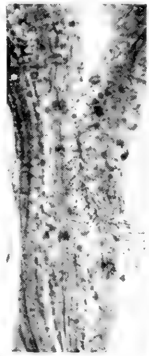

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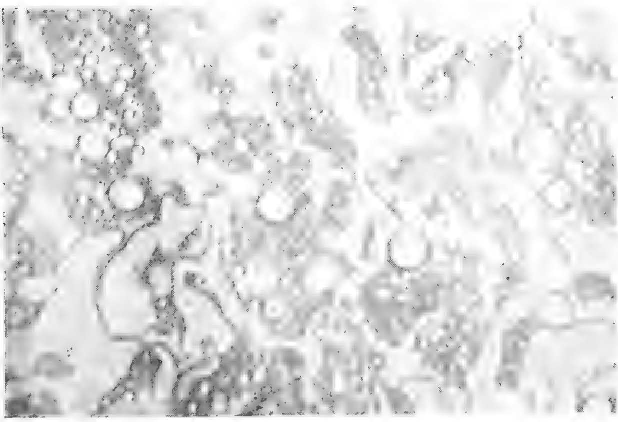

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PLATE IV.

Fig. 1.—Oral cilia of Stentor.

Fig. 2.—Section of stentor at the point of attachment of the oral cilia showing their attachment to the body.

Fig. 3 and 4.—Teased myonemes of stentor showing their fibrillar nature.

Fig. 5.—Myonomes of Stentor.

Fig. 6.—Contractile stalk of Vorticella. Photographed by Dr. C. F. Hodge with Ultraviolet microscope.

Fig. 7.—Ultraviolet microphotograph of striped muscle. Photographed by Dr. C. F. Hodge.

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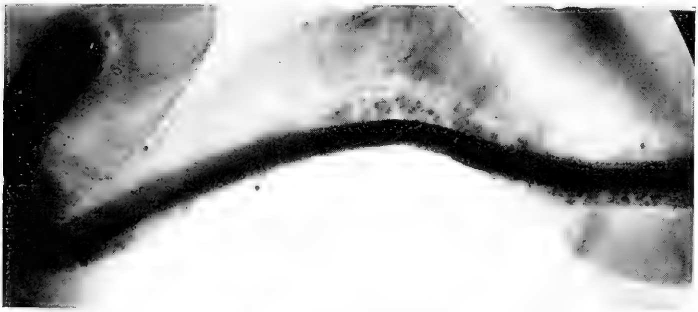

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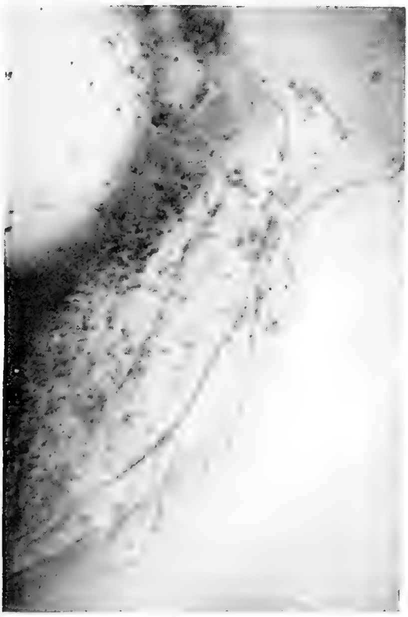

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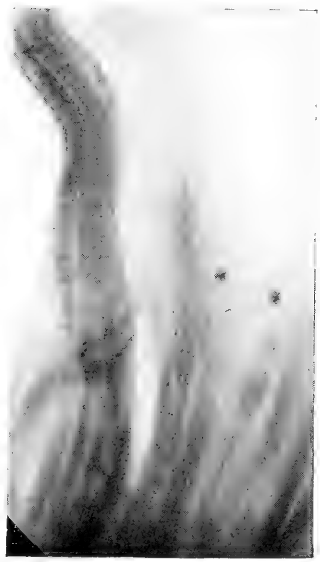

7

THE DEVELOPMENT OF HOLOTHURIA FLORIDANA POURTALÉS WITH ESPECIAL REFERENCE TO THE AMBULACRAL APPENDAGES.

BY

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CONTENTS.

I. Introduction.

During the summer of 1888, at Green Turtle Cay, Bahamas, I studied the ontogeny of Holothuria floridana Pourtalés. For the identification of the species I relied at that time upon the labelled specimen in the Museum of the Biological Department of the Johns Hopkins University, and thus in my preliminary notice (1889) the form appeared under the name of Mülleria agassizii Selenka. In recent papers (1905, 1908) I have shown that this sea-cucumber, common from Florida through the Bahaman, West Indian, and Caribbean regions to the northern coast of South America, is Holothuria floridana Pourtalés, with which Holothuria mexicana Ludwig, and Holothuria africana Théel, are identical. In addition I have established the fact that Holothuria atra Jäger, generally distributed throughout the Indo-Pacific regions, is a distinct species, and I defined for each of these species the differential characters, some of which had not been previously described.

Holothuria floridana is found in large numbers in the shallow bays and sounds on either the white coralline sand, or more generally where the bottom is covered with green and brown vegetation. These holothurians, sometimes uniformly seal-brown in color, but more frequently particolored in varying mixtures of browns, creams, and grays, are well protected both by their coloration, and by the habit of covering themselves more or less completely with pieces of plants, shells and sand held fast by the suckers of the pedicels.

This species breeds during July and August, albeit some individuals may be found with mature gonads both before and after this season. A number of attempts to artificially fertilize the eggs failed. The live-box method employed by Selenka, 1876, was very successful. About one hundred of the holothurids were collected within an hour or two, and placed in a large box, the cracks of which had been covered with cheese-cloth. The box was anchored to the bottom in a shallow bay where at low tide the sea-water barely covered it. In from four to ten hours eggs and sperms were extruded. The oosperms, heavier than water, sank to the bottom and were gathered through a rubber tube into shallow glass dishes. Four lots of oosperms were obtained in the summer of 1888, but in two succeeding Bahaman expeditions I have not been able to secure the embryos.

Holothuria floridana does not develop a free Auricularia larva, but the embryonic stages are passed within the vitelline membrane during the first five days after fertilization of the egg. On the sixth day the embryo hatches as a larva with five primary tentacles, four developed and one as a bud, and also with one posterior pedicel.

In my study of the order of development of the tentacles, pedicels and papillæ, embryos of each stage were reconstructed by plotting the serial sections on paper. In this manner appendages were found that could not be seen from a surface view of the whole embryo, and the exact origin of each appendage from one side, or the other, of a given radial canal, was determined. The interpretation of the origin of an appendage based upon a surface view is sometimes misleading, for the ambulacral canal may grow around in the body-wall, pass over the radial canal and thus the appendage will emerge from the skin upon the opposite side from which it leaves the radial canal.

In my preliminary communication (1907), I had not completed this study by reconstruction from serial sections and thus a few discrepancies were then published. I have called all appendages *developed,* which clearly project from the skin and seem to be functional, even if much contracted and very short. Those still buried in the body-wall, often merely initial evaginations from the radial canal, and then determinable only in sections, I have called *buds*.

II. Notes on the General Embryology.

The formation of the polar bodies and fertilization are followed by a total and approximately equal cleavage. The stage with four blastomeres is reached at three hours, with sixteen blastomeres at four hours and the blastula by the fourteenth hour. Then the formation of mesenchyme begins by cell-proliferation at the vegetative pole, while at the same time gastrulation takes place. By the twenty-second hour a plug of cells has grown out toward the blastopore, from the blind end of the archenteron, dividing this sac into two diverging diverticula of which the ventral constitutes the enteron, and the dorsal, the vaso-peritoneal vesicle. In the second day the vaso-peritoneal vesicle grows larger and begins to show the division into hydrocele and enterocele (Pl. I, Fig. 2). During this day a crescentic depression on the ventral surface marks out the initiation of the peristome (Pl. I, Fig. 1). This depression gradually deepens and straightens, growing out to either side until it extends entirely across the ventral surface of the embryo (Pl. I, Fig. 3). The plane of the peristomial groove is at an angle of fifty degrees with the sagittal plane of the adult holothurid.

At this time spots of green pigment appear in groups, but later are evenly distributed over the whole surface. Thus the brown embryo gradually becomes greenish in color. During the second and third days the ectoderm is ciliated and the embryo revolves within the vitelline membrane.

At the time of hatching the mouth has become established medianly in the peristome, while the enteric canal has the characteristic dorsal, left, and right loops (Pl. II-III, Figs. 7-14). As the young holothurid creeps about, it begins to eat the protoplasmic fragments in

the slime and the living organisms which have been allowed to remain on the bottom of the dish and form a culture for food when the stale water has been decanted at each period. As the young animal feeds, the mid-enteron enlarges into the prominent larval stomach, occupying the middle third of the cœlom. By the ninth day the divisions of the enteric canal are clearly shown. The first or dorsal loop is suspended by its mesentery from the mid-dorsal interradius. It includes the œsophagus and stomach, and terminates ventrad of the anus. From the posterior end of the stomach the intestine turns down in the left ventral interradius, and runs forward as the second or left loop. This portion gradually goes over to the right ventral interradius, until just in front of the stomach, where it turns again posteriorly, and as the third, or right loop, goes in the right ventral interradius to the posterior end of the stomach. Here the intestine makes a sharp bend dorsad along the right side of the stomach, and terminates in the anus, which is now near the posterior end of the mid-dorsal region of the body. The large larval stomach thus crowds the second loop ventrad alongside of the third loop, and, at the same time, the large Polian vesicle lying in the left half of the cœlom, pushes the first loop of the enteron to the right. This position is maintained together with the relatively large size of the stomach, in a general way, during the developmental stages of my series, but following about the fortieth day the second loop comes gradually dorsad toward its adult position.

The stone-canal has a peripheral expansion, the madreporic vesicle, similar to that described in Cucumaria planci by Ludwig, 1891. In Holothuria floridana the stone-canal is found in the embryo of four days. Later it lies in the dorsal mesentery and the madreporic vesicle is at the surface in the mid-dorsal line of the median plane. The outer wall of the vesicle at first is continuous with the surface of the body-wall, but later the vesicle lies deeper in toward the cœlom. In Cucumaria planci, Ludwig says that the dorsal pore is obliterated in eighteen to twenty days, and that the madreporic vesicle opens into the cœlom on the ninety-eighth day. In Holothuria floridana, in the sixth day, the dorsal pore is not open at the surface, and in my oldest stage, eighty-seven days, the madreporite is still continuous with the tissues of the body-wall.

By the time of hatching, the Polian vesicle has arisen from the circular canal in the left *ventral* interradius. It is well marked in the seventh day, and by the ninth, extends posteriorly one-half the length of the cœlom. Joh. Mühler, 1852, in the "Auricularia mit Kugeln" and Ludwig, 1891, in Cucumaria planci, describe the origin of the Polian vesicle in the left *dorsal* interradius. However, Thompson, 1862, in Synapta inhærens, states that the Polian vesicle arises in the left ventral interradius, as I have found it in Holothuria floridana.

The Polian vesicle enlarges rapidly and nearly fills the left half of the cœlom. By the sixty-seventh day it extends three-fourths of the way to the posterior end of the body. During this time the anlagen of the radialia and interradialia of the calcareous ring are being established.

From observation of the living holothurids it is apparent that the respiratory movements of the cloaca begin quite early in the free larvæ. However, sections of my stages do not clearly reveal the presence of *respiratory trees* until the fortieth day, although at that time they are well developed, the left being the larger. Hence, it is probable that they arise in an earlier stage. In some cases only one respiratory tree is to be found. The radiating cloacal dilator muscles, which cause the respiratory movements of the cloaca, are also well established at an early date.

On the third day, the anlagen of the first calcareous spicules appear. Beginning with a simple short rod, the ends bifurcate to form a four-rayed base. Sometimes only three rays are developed. The outer ends of the rays fork. From the central part of the bar arise the four vertical rods which are joined together by cross-beams to form the spire. The ends of the branches of the rays again fork, and the apposed terminal branches from neighboring rays grow together to form the four central holes of the disk. Then the circle of peripheral holes is developed in the same manner. The rosettes and perforated plates are formed just as the disks of the tables.

In the tentacles, along with the tables and perforated plates, by the fourteenth day, circular and spiral supporting rods are developed. The spiral rods extend from three-fourths to one, two, or even three

turns around the tentacle. At the base of the sucker is a supporting ring giving off branches running out at right angles into the wall of the sucker. Guarding the anus are two lateral and one posterior, broadly based, fan-shaped perforated plates (Pl. I, Fig. 6; Pls. II-III, Figs. 9, 10, 12, 14, vv, y) which wave in and out with the contractions of the cloacal muscles. I have interpreted these as structures similar to vestigial anal teeth (1909).

III. Tentacles.

In the fourth day, four of the five primary tentacles grow out from the bases of the radial canals, two before and two behind, the peristomial groove. During this day the tentacles extend, pushing ahead of themselves the overlying ectoderm of the peristome, together with the intervening mesenchyme. The lip of the peristome, divided by the groove into anterior and posterior halves, is moved in and out by the contractions of the developing tentacles within. At this time four of the primary tentacles are well marked (Pl. I, Figs. 4-5), one arising from the mid-ventral radial canal to the right (MVr1), and one dorsad from the right (RVd1) and left ventral (LVd1) and the left dorsal (LDd1) radial canals respectively. In addition, the *bud* of the fifth primary tentacle appears, arising from the mid-ventral radial canal to the left. Thus the four-day embryo presents a well marked tentacle in each interradius except the right ventral, which, however, contains the bud of the fifth primary tentacle. Since MVr1 and LDd1 are larger than RVd1 and LVd1 (Pl. I, Fig. 4), it is probable that the two former arise first. The ventral radial canal has grown much faster than the others and ends posteriorly in the bud of the first pedicel (Pl. I, Figs. 4-5, pvp).

During the fifth day, while the embryo is still within the vitelline membrane, the tentacles grow but slightly. At this time the tentacles contract comparatively rapidly. They push out against the vitelline membrane now covered on the outside by slime, which contains diatoms and other protists and their debris. Once in a while a tentacle adheres to the unbroken egg-shell, pulling it in. By the sixth day the embryo has succeeded, by constant manipulation with the tentacles, in breaking the vitelline membrane. The

larval holothurid gradually pushes away the remnants of the old egg-shell and begins to creep about by means of the suckers terminating the four primary tentacles first developed, and the one posterior mid-ventral pedicel. It will fix itself by the pedicel and extend the tentacles in hydra-like attitude (Pl. II, Fig. 7). Now it will turn onto the tentacles with the released pedicel waving aloft, and again it will creep about by means of all of its appendages, assuming curious elephantine postures. Securing food by the suckers, the holothurid bends the tentacles until the ends reach the mouth, when the food is pushed into the pharynx. The outer lip of the peristome embraces the proximal halves of the tentacles, forming a web between them which is drawn out when the tentacles are extended.

During the fourth, fifth and sixth days, the fifth primary tentacle remains as a bud from the mid-ventral radial canal to the left. By the seventh day the bud has appeared externally as a small tentacle and with the exception of an occasional precocious individual it is not until after the seventh day that the fifth primary tentacle develops to the size of the first four (Pl. II, Fig. 7, MVl1). The lip of the peristome persists as a collar around the bases of the tentacles.

In the scheme for the adult symmetry of the twenty tentacles (Diagram 1), the origin of the thirteen shown in my series is indicated. Each of the first four of the primary tentacles, so well inaugurated in the fourth day, is marked A, and the fifth, budding in the fourth day, A5. Following this scheme of symmetry, Table I present the details in the development of the tentacles from the sixth to the eighty-seventh days. After the seventh tentacle, the order of appearance sometimes varies, but the serial numbers, given in Diagram 1, follow the usual ontogeny.

By the fortieth day, the sixth tentacle, small but developed (Pl. II, Fig. 10, RVd2) is clearly seen in the living holothurid. It has evaginated from the tentacular canal of the primary tentacle dorsad from the right ventral radial canal just after it leaves the radial canal (Diagram 1, R 6). One specimen of this age has also the bud of the seventh tentacle from the canal of the primary tentacle dorsad from the left ventral radial canal (L 7). The day when the bud of the sixth tentacle may have first appeared, I was not

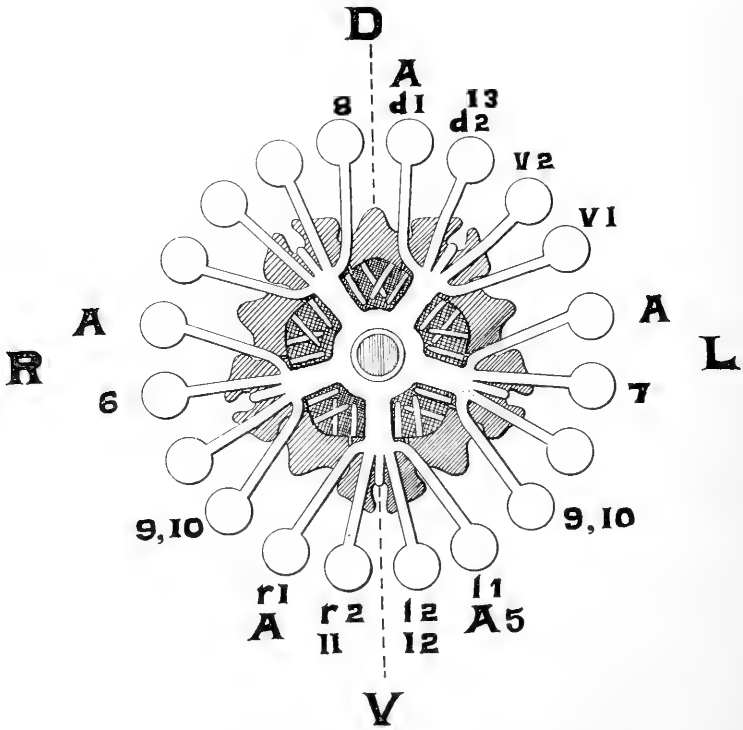

Diagram I. Order of development of thirteen of the twenty tentacles constituting the adult symmetry. The vertically hatched central oesophagus is surrounded by the circular canal of the water-vascular system, from which the five radial canals arise. The five radialia, and the five interradialia, are hatched diagonally. At the base of each radiale, the radial canal gives off two tentacular canals to each side and then proceeds anteriorly, turning through the notch into the body-wall. The first tentacular canal to be developed runs along the inner surface of the radiale-interradiale joint and the second, having been evaginated from the base of the first, goes alongside of the radial canal. At the anterior margin of the calcareous ring, the tentacular canals give off the ampullæ and then terminate in the peltate tentacles. The ampullæ hang down behind the calcareous pieces, and, as they come into view, are seen outlined against the cross-hatching which represents the cœlom.

D. Dorsal. V. Ventral. R. Right. L. Left. d1, d2, v1, v2. Tentacles dorsad and ventrad respectively, in the order of development from a given radial canal. r1, r2, l1, l2. Tentacles to the right, and to the left, respectively, in the order of development from the mid-ventral radial canal. A. One of the first four primary tentacles. A5. The fifth primary tentacle. 6, 7, 8, 9, 10, 11, 12, 13. Secondary tentacles in serial order of development.

TABLE I.—Order of Development of Tentacles in Holothuria Floridana.

Number of Individuals.	Age in Days.	Radial Canal.																				Serial Number in Development of Secondary Tentacles.							
		Mid-Ventral.				Right Ventral.				Left Ventral.				Right Dorsal.				Left Dorsal.											
		r1	r2	l2	l1	d1	d2	v2	v1	d1	d2	v2	v1	d1	d2	v2	v1	d1	d2	v2	v1	6	7	8	9	10	11	12	13
	4	A			b	A				A								A											
	5	A			b	A				A								A											
	6	A			b	A				A								A											
4	7–8	A			s	A				A								A											
73	7–33	A			A	A				A								A											
1	40	A			A	A	s			A	b							A				s	b						
1	40	A			A	A	s		b	A	b		b	b				A				s	b	b	b	b			
1	42	A			A	A	s			A	b			b				A				s	b	b					
1	45	A			A	A	s			A	s			b				A				s	s	b					
1	49	A	b		A	A	s			A	s		b	s				A				s	s	s	b		b		
1	51	A			A	A	s		b	A	s		b	s				A				s	s	s	b	b			
1	53	A			A	A	D		s	A	D		s	D				A				D	D	D	s	s			
1	55	A			A	A	D		b	A	D		b	s				A				D	D	s	b	b			
1	67	A	b		A	A	D		s	A	D		s	s				A				D	D	s	s	s	b		
1	71	A		s	A	A	D		b	A	D		b	s				A				D	D	s	b	b		s	
1	75	A			A	A	D		s	A	D		s	D				A				D	D	D	s	s			
1	75	A			A	A	D		s	A	D		s	D				A	D			D	D	D	s	s			D
1	87	A	s		A	A	D		b	A	D			s				A	s			D	D	s	b		s		s

Table I is arranged in accord with the scheme of symmetry given on p. 218. r1, r2, l2, l1. Tentacles to the right, and to the left respectively, from the mid-ventral radial canal. d1, d2, v2, v1. Tentacles dorsad, and ventrad, respectively, from a given radial canal. A. One of the five primary tentacles. b. Bud of a tentacle. s. Small tentacle. D. Developed tentacle.

able to determine because of the lack of stages between the thirty-third day, with no trace of the sixth tentacle, and the fortieth day with the sixth developed and the seventh budded. A second forty-day specimen, showing precocious development, has in addition, the buds of the eighth tentacle dorsad from the right dorsal radial canal (D 8) and the ninth and tenth (R and L, 9, 10), ventrad from the right and left ventral radial canals. Thus for the first time, at the fortieth day, is a tentacle (D8) given off from the right dorsal radial canal, although so early as the twenty-fourth day this canal has evaginated a papilla. In some specimens the ninth tentacle first appears on the left, in others, on the right, but in most cases these two appear at the same time. In the forty-second day the eighth tentacle is found externally (Pl. III, Figs. 11-14, RDd1). A forty-nine day specimen presents the bud of the eleventh tentacle from the primary tentacular canal to the right of the mid-ventral radial canal (Diagram 1, V 11) one at seventy-one days shows a small tentacle, the twelfth, from the canal of the primary tentacle to the left of the mid-ventral radial canal (V 12), and one at seventy-five days, the thirteenth (D 13), from the primary tentacular canal dorsad from the left dorsal radial canal (Pl. III, Figs. 13-14, LDd2). Thus, in the development of the adult symmetry, as represented in Diagram 1, by the end of my ontogenetic series, the following tentacles have appeared, albeit not all in any one holothurid; the four from the mid-ventral radial canal, the two dorsal and one of the ventral from each of the lateral ventral radial canals, the two dorsal from the left dorsal radial canal and only one from the right dorsal radial canal. In general, beginning with the fifth, the tentacles develop in alternation from left to right.

By the eighth day the suckers of one or more of the tentacles are divided. Later these halves divide and the dichotomous branching of the adult tentacle is established. (Pls. II-III, Figs. 7-14).

By the sixty-seventh day the tentacle ampullæ have become developed and can be followed in cross-sections from their place of origin from the bases of the tentacular canals at the anterior end of the calcareous ring to about one-half the distance back to the circular canal. The origin of the tentacle ampullæ does not seem to have been previously noted.

A. Discussion of Literature.

Kowalevsky, 1867, describes in Cucumaria kirchsbergii and Cucumaria planci and Selenka, 1876, also in Cucumaria planci, the formation, at first of three dorsal, and then later, of two ventral tentacles. Ludwig, 1891a, states that in Cucumaria planci and, 1898, in Phyllophorus urna and Clark, 1898, that in Synaptula hydriformis, all of the five primary tentacles appear at the same time. Ludwig, 1891, also describes in Cucumaria planci an asymmetrical development of the tentacles but of a somewhat different pattern from that which I find in Holothuria floridana. In agreement with my results, Ludwig shows that two of the five primary tentacles are given off from the mid-ventral radial canal, one to either side, but, of the three others, two arise from the left dorsal radial canal, dorsad and ventrad respectively, and one ventrad from the right dorsal radial canal. Thus each of the right and left ventral radial canals gives rise to a primary tentacle in Holothuria floridana and does not in Cucumaria planci. Up to this time the determination of the exact origin of the tentacles from definite radial canals has been made only in Cucumaria planci and Phyllophorus urna by Ludwig and in Holothuria floridana by myself, with the above divergent findings. When other holothurians are studied in a similar manner, it will be of interest to see whether these differences are generic, or specific, and if yet other such peculiar asymmetrical distributions of the tentacles prevail. So it would appear that the generalization of Becher, 1907, 1908, that in addition to the mid-ventral radial canal the left, and to a less degree the right dorsal radial canals were the more important in the primitive ancestral holothurian, is scarcely justifiable at present. The simple primitive pedicel-like structure and function of the tentacles in the early larvæ of Holothuria floridana is like that described by Becher, 1907, in the adult Rhabdomolgus ruber.

Relative to the increase in number of the tentacles beyond the primary five, we have but few observations. Danielssen and Koren, 1856, describe the appearance of distinct traces of five new tentacles in Holothuria tremula on the forty-seventh day. In nine days these buds grow to about the full size of the primary tentacles. In view

of the results obtained by Ludwig and myself, it is much to be desired that Holothuria tremula be reinvestigated through the careful study of serial sections of each stage.

Becher, 1907, describes the simultaneous appearance of the three first secondary tentacles in the three dorsal interradii of Rhabdomolgus ruber. Thompson, 1862, merely states that in Leptosynapta inhærens, at about three months, the sixth and seventh tentacles arise on opposite sides of the circular canal. Baur, 1864, with equal lack of exactness, tells of an eight tentacled stage in Labidoplax digitata, as intermediate between the primary five, and the adult twelve, tentacles. Clark, 1898, notes the first five accessory tentacles as appearing at the same time in Synaptula hydriformis and agreeing in location with those of Cucumaria planci as described by Ludwig. The eleventh tentacle arises ventrad from the left, and the twelfth, ventrad from the right dorsal "secondary outgrowth." While Clark does not suggest the homology, it is possible to regard these "secondary outgrowths" as the last vestiges in the degeneration of the protoholothuroid radial canals, thus supporting the phylogenetic theories of Ludwig, 1889-92, and Oestergren, 1907. Ludwig, 1891, does not note an increase in the number of tentacles in Cucumaria planci until the hundred and sixteenth day, when some of the young animals have the sixth and seventh tentacles dorsad of the right and left ventral radial canals. On the sixteenth day the primary tentacles develop branches and inaugurate the arborescent form. The same author, 1881, observes that in young Chiridota rotifera, 1898, in Taeniogyrus contortus, and, 1898a, in Phyllophorus urna, two secondary tentacles have arisen, one dorsad from each of the right and left ventral radial canals. Semon, 1883, believes that, in Labidoplax digitata, the secondary tentacles will prove to be evaginated from the radial canals, at the point where the latter bend over the calcareous ring. Semon emphasizes that his conclusion is theoretical, and not based on direct observation.

IV. Pedicels and Papillae.

As related above, in Holothuria floridana the first pedicel is found as a bud terminating the posterior end of the mid-ventral radial

canal in the fourth day (Pl. I, Figs. 4-5, pvp). After hatching in the sixth day, this pedicel develops a sucker, and by the eighth day it exceeds the tentacles in length. On the seventh day, the bud of the second pedicel evaginates from the mid-ventral radial canal to the left. On the ninth day this pedicel appears externally (Pl. II, Fig. 7, b). On the twenty-second day the third mid-ventral pedicel arises between the first and second, and on the thirty-third day, the fourth, behind the third (Pl. II, Fig. 8, c, d). On the fortieth day, the fifth mid-ventral pedicel appears behind the fourth (Pl. II, Fig. 10, e). After the primary posterior mid-ventral pedicel, the next four develop to the left from the mid-ventral radial canal. *It is not until the fortieth day* that the bud of a pedicel appears *to the right* from the mid-ventral radial canal (Pl. II, Fig. 10, MVrp). The ventral pedicels are thus inaugurated in an asymmetry beginning with development to the left of the mid-ventral radial canal. In one lot of embryos of which I have now only sketches from life, the development was more rapid than given above, since a specimen of the fourteenth day has three, and one of the nineteenth day, four, left mid-ventral pedicels.

In Table II, the appendages of the young of my alcoholic series, from four to eighty-seven days old, are seriated with reference to their place of origin. In the stages later than nine days, variation in the number of appendages will be noted in the individuals. In part, this is to be associated with accelerated or retarded growth, and in part, with natural variation. As noted above in connection with the tentacles, at least in one lot of embryos, individuals developed more precociously.

On the twenty-fourth day, the buds of the first pair of papillæ appear ventrad from the anterior parts of the dorsal radial canals, inaugurating the bilateral symmetry later shown, in a general way, in the distribution of the appendages. By the thirtieth day these buds have developed, and by the thirty-third day the buds of five more dorsal papillæ have appeared (Pl. II, Fig. 9). From the first, these papillæ are especially developed and prominent (Pls. II-III, Figs. 9, 10, 12, 14; rdpa 1, 2, 3, ldpa 1, 2, 3). Later the ventral series of the dorsal papillæ become the lateral warts so characteristic

TABLE II.—DISTRIBUTION OF PEDICELS AND PAPILLÆ IN HOLOTHURIA FLORIDANA.

Number of Individuals.	Age in Days.	Mid Ventral Radial Canal. To the Right. Dv *	Mid Ventral Radial Canal. To the Right. Bd *	Mid Ventral Radial Canal. In Radius. Dv	Mid Ventral Radial Canal. In Radius. Bd	Mid Ventral Radial Canal. To the Left. Dv	Mid Ventral Radial Canal. To the Left. Bd	Radial Canal. Right Ventral. Ventrad. Dv	Radial Canal. Right Ventral. Ventrad. Bd	Radial Canal. Right Ventral. In Radius. Bd	Radial Canal. Right Ventral. Dorsad. Dv	Radial Canal. Right Ventral. Dorsad. Bd	Radial Canal. Left Ventral. Ventrad. Dv	Radial Canal. Left Ventral. Ventrad. Bd	Radial Canal. Left Ventral. In Radius. Bd	Radial Canal. Left Ventral. Dorsad. Dv	Radial Canal. Left Ventral. Dorsad. Bd	Radial Canal. Right Dorsal. Ventrad. Dv	Radial Canal. Right Dorsal. Ventrad. Bd	Radial Canal. Right Dorsal. In Radius. Bd	Radial Canal. Right Dorsal. Dorsad. Dv	Radial Canal. Right Dorsal. Dorsad. Bd	Radial Canal. Left Dorsal. Ventrad. Dv	Radial Canal. Left Dorsal. Ventrad. Bd	Radial Canal. Left Dorsal. In Radius. Bd	Radial Canal. Left Dorsal. Dorsad. Dv	Radial Canal. Left Dorsal. Dorsad. Bd	Total. Dv	Total. Bd	Total. Dv and Bd	Ratio—Trivium to Bivium.
	4				1																								1	1	
	5				1																								1	1	
12	6–16			1																								1		1	
3	7–13			1			1																					1	1	2	
53	9–24			1		1																						2		2	
4	22			1		1	1																					2	1	3	
1	22			1		2																						3		3	
1	24			1		2													1					1				3	2	5	1.50
1	30			1		2												1					1					5		5	1.50
1	30			1		3												1					1					6		6	2.00
1	30			1		2			1					1				1					1					5	2	7	2.50
1	33			1		3		1					1					1	1	1		1	1	1	1			8	5	13	.86
1	40		1	1		3		1	2				1	2				1		1		1	1		1		1	8	9	17	1.83
1	40		1	1		4		1	2				1	2				1	2	1		3	1	2	1		2	9	16	25	.92
1	42		1	1		4		2	1			1	2	1			1	2	2	1		2	2	1	1		4	13	16	29	.93
1	45	1	2	1		5		4	1			6	4	3			3	3	1	1	1	4	3	2	1	1	3	27	23	50	1.50
1	49	1	2	1		3	1	4	1		1	2	4			1	3	3	1	1	1	3	3	2	1		4	22	21	43	1.26
1	51	1	2	1		4	1	3	2	1		4	3	2	1		3	2	3	1	1	4	2	4	1	1	5	18	34	52	1.17
1	53	4	3	1		4	3	5	1			7	5	1			7	3	7	1	1	9	3	6	1	1	12	27	58	85	.93
1	55	2	1	1		4	2	5	3		2	3	5	2		2	3	4	2		4	3	4	2		3	5	36	26	62	1.30
1	67	2	1	1		4	4	5	1			5	6	1			7	3	6	1	1	8	3	6	1	1	8	26	49	75	.97
1	71	4	1	1		5	2	4	3	1		7	5	2	1		8	3	5	1	2	7	3	5	1	2	7	29	51	80	1.22
1	75	2	5	1		3	5	5	2		1	7	4	2		2	6	3	10		2	8	3	7		1	10	27	62	89	1.02
1	75	4	2	1		4	2	6	3	1		12	5	4	1		8	4	8	1	1	9	4	5	1	1	11	30	68	98	1.18
1	87	2	3	1		4	2	3	3	1		5	4	1	1		6	2	4	1	2	4	2	4	1	1	5	21	41	62	1.39

of the adult Holothuria floridana (Edwards, 1908). However, most of the dorsal appendages will become pedicels since in the adult only about twenty per cent are papillæ.

On the thirtieth day a pedicel buds ventrad from the right and left ventral radial canals. By the thirty-third day these lateral pedicels have developed (Pl. II, Fig. 8, rvp, lvp), and by the fortieth day two additional buds have appeared below each lateral ventral radial canal (Pl. II, Fig. 10). On the forty-second day one bud arises dorsad from each lateral ventral radial canal. From this time on the number of appendages increases with comparative rapidity and an approximate bilateral symmetry is established (Pl. III, Figs. 11-14). For the details of distribution I refer to Table II.

In one specimen of the fortieth day, nine developed appendages and sixteen buds have appeared; by the forty-fifth day, twenty-seven developed and twenty-three buds; by the fifty-fifth day, thirty-six developed and twenty-six buds and by the seventy-fifth day a total of ninety-eight (Pls. II-III, Figs. 10-14). The largest number of developed appendages, thirty-six, is found in a fifty-five day holothurid, while among the smallest specimens of my collection not raised from the embryo, the least number of developed appendages shown is seventy-seven. If studied in sections, these small individuals would doubtless be found to have buds and show a considerable increase in the total number of appendages. Since the seventy-five day young presents a total of ninety-eight appendages, developed and buds, it may be assumed that the later stages in my holothurids from the embryo, connect this series with the adult and that appendages will continue to bud from each radial canal in a manner similar to that described up to the eighty-seventh day.

There is a tendency, perfectly demonstrated in part of the specimens, for one or another radial canal to terminate posteriorly in the bud of an appendage, either lying in the radius, or turned slightly dorsally or ventrally. As the radial canal grows on posteriorly, such a terminal appendage becomes either ventral, or dorsal, to the canal in position. At first the ordinary larval appendages grow off to the sides of the radial canals and, hence, the lines of the radii are bare. This is especially true of the mid-ventral radius,

and may characterize the young of the usual collection as Pourtalès, 1851, notes. The ambulacral vessels of a number of the appendages, particularly in the later stages, as may be seen in the sections, grow within the body-wall for some distance from the radial canal and consequently the appendage is superficially interradial in location. On the other hand, the ambulacral vessels of some appendages turn into the mid-line of the radius. Hence, through the varying lengths of ambulacral vessels within the body-wall the fairly even distribution of appendages over the trivium, and in smaller numbers, over the bivium, of the adult is established.

From the larva of twenty-four days, with the first dorsal papillæ, to the eighty-seven day stage, there are eighteen cases detailed in Table II, of which seventy-two per cent have the larger number of appendages from the radial canals of the trivium. In making the ratio of appendages in the trivium to those in the bivium, the appendages given off dorsad from the right and left ventral radial canals were counted among those of the trivium, with which they seem to function, albeit some of them later in the adult might be placed in the bivium. The average of all the ratios gives one and three-tenths as many appendages in the trivium as in the bivium. The proportionate number ventrally increases with further growth, for, as I have demonstrated elsewhere (1908), in the average young of the ordinary collection series there are one and seven-tenths, and in the average adult, one and nine-tenths, as many appendages per square centimeter in the trivium as in the bivium. Since, on the average, in this series, there are half again as many appendages per square centimeter in the young as in the adult the increase in the number of appendages does not keep pace with the general growth of the body-wall. In every stage from larva to adult, all of the ventral appendages are pedicels. Of the dorsal appendages of the adult, eighty per cent are pedicels, and not all of the remaining twenty per cent are true papillæ. In each ambulacral appendage a valve develops at the entrance by the time the appendage is functional. The last stages of my series fail to exhibit any clearly marked ampullæ for the pedicels and papillæ. The relatively large Polian vesicle, therefore, functions as a general reservoir for the larval ambulacral system.

A. Discussion of Literature.

Joh. Müller, 1853, reports the first pedicel in the "Auricularia mit Kugeln," as from the mid-ventral radial canal posteriorly and to the right. Previous to my work (1889, 1907) all other authors, Krohn, 1851, in Cucumaria planci; Danielssen and Koren, 1856, in Holothuria tremula; Agassiz, 1864, in Psolus fabricii; Kowalevsky, 1867, in Cucumaria kirchsbergii, Cucumaria planci and Phyllophorus urna; Selenka, 1876, and Ludwig, 1891a, in Cucumaria planci, and Ludwig, 1898, in Phyllophorus urna, describe the first two pedicels as developing in a pair from the posterior end of the mid-ventral radial canal. Ludwig notes the origin of the first pair in the fourth day and that these pedicels are truly developed on the eighteenth day. Danielssen and Koren, 1856, record in an embryo of Holothuria tremula of the thirty-fourth day, the appearance of the second pair of pedicels above the first, and on the fifty-sixth day, the third pair. In the last stage also, dorsal papillæ are seen here and there. Ludwig, 1891, describes in Cucumaria planci, the third pedicel arising to the left of the mid-ventral radial canal, in front of the two primary, on the forty-fifth day. The fourth pedicel does not appear until the eighty-fourth day and then still further forward, but to the right. The fifth pedicel arises on the hundred and eleventh day anteriorly and ventrad from the left dorsal radial canal. Therefore, the same radial canals from which the primary tentacles developed serve for the budding of the first pedicels. Ludwig, 1898, states that in Phyllophorus urna the third pedicel appears before, and the fourth pedicel behind the first two. In this fashion a zigzag line is formed. At two and one-half months the larva possesses two more mid-ventral pedicels, and two from each lateral ventral radial canal, but none in the bivium.

Becher, 1908, makes various suggestions as to the possible phylogenetic significance of this marked precocity in the ontogeny of the pedicels from the mid-ventral radial canal, without, however, coming to any satisfactory conclusion. Because the appendages increase with growth, Hérourard, 1901, believes that a single species passes successively through stages characteristic of Ocnus, Cucumaria and Semperia, and, therefore, the first and last are synonyms of Cucumaria.

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EXPLANATION OF PLATES.

All figures were drawn with the aid of an Abbé camera lucida, from preparations of Holothuria floridana Pourtalés.

ABBREVIATIONS.

a, b, c, d, e, first, second, third, fourth and fifth pedicels from the mid-ventral radial canal.

bl., blastopore.

d1, d2, first, and second, tentacles developed dorsad from a given radial canal.

D., dorsal.

ect., ectoderm.

ent., enteron.

l1, l2, first and second tentacles developed to the left from the mid-ventral radial canal.

LD., left dorsal radial canal.

ldpa1, ldpa2, ldpa3, first, second, and third, left dorsal papillæ.

lvp., first pedicel developed ventrad from the left ventral radial canal.

LV., left ventral radial canal.

m., mouth.

mes., mesoderm

MV., mid-ventral radial canal.

MVrp., first pedicel developed to the right from the mid-ventral radial canal.

p., peristome.

pvp., posterior ventral pedicel.

r1, r2, first and second tentacles developed to the right from the mid-ventral radial canal.

RD., right dorsal radial canal.

rdpa1, rdpa2, rdpa3, first, second, and third, right dorsal papillæ.

rvp., first pedicel developed ventrad from the right ventral radial canal.

RV., right ventral radial canal.

t., larval table.

V., ventral.

v1, v2, first, and second, tentacles developed ventrad from a given radial canal.

vm., vitelline membrane.

vpv., vaso-peritoneal vesicle.

vv., lateral anal plates.

xx., anterior papillæ, developed dorsad from the right and left dorsal radial canals.

y., posterior anal plate.

EXPLANATION OF PLATE I.

FIGS. 1, 3, 4, and 5. Surface views of embryos, represented as opaque objects within the vitelline membrane. The nuclei of the ectoderm cells, and the scattered mesenchyme cells beneath, are indicated.

FIG. 1. Posterior view of forty-four hour embryo, showing the peristome in front of the blastopore and the vaso-peritoneal vesicle within. $\times$ 175.

FIG. 2. Sagittal section of embryo represented in Fig. 1. The vaso-peritoneal vesicle is divided into anterior hydrocele and posterior enterocele. The enteron is growing toward the bottom of the peristome. $\times$ 175.

FIG. 3. Ventral view of embryo showing the peristome as a straight groove across the ventral surface. $\times$ 175.

FIG. 4. Ventral view of an embryo of four days. The four of the primary tentacles first developed and the bud of the posterior mid-ventral first pedicel, are well marked. $\times$ 175.

FIG. 5. Embryo represented in Fig. 4, seen from the right side. $\times$ 175.

FIG. 6. One of the three calcareous perforated anal plates. $\times$ 135.

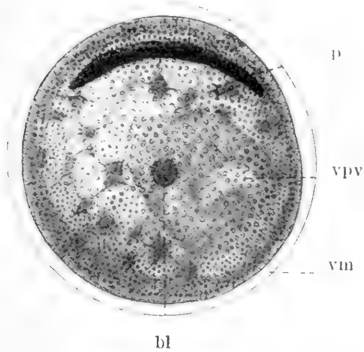

FIG. 1.

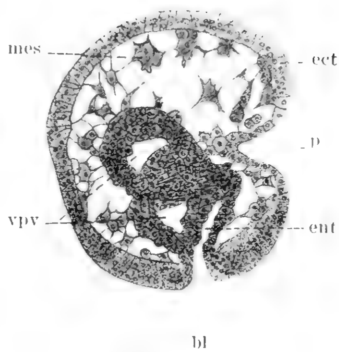

FIG. 2.

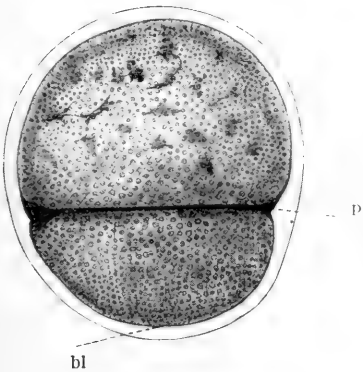

FIG. 3.

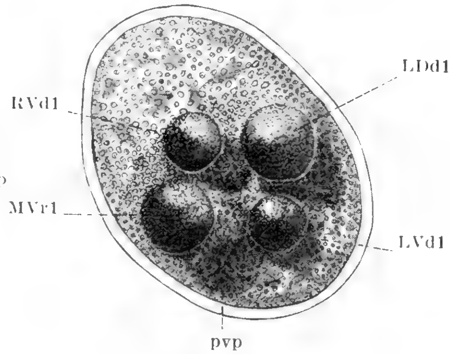

FIG. 4.

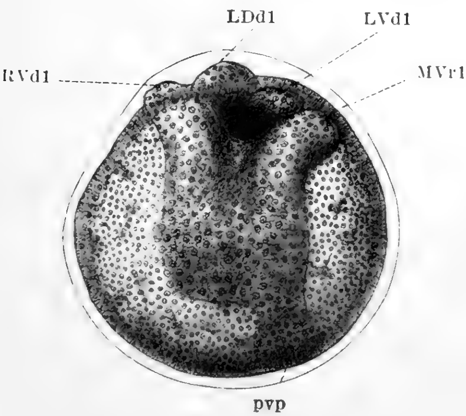

FIG. 5.

FIG. 6.

PLATE II.

Figs. 7, 8, 9, 10 show the larval tables (t), the terminal branching of the tentacles, the radial canals and the enteron, with its enlarged stomach and three loops.

Fig. 7. Ventral view of a larva of nine days showing the five primary tentacles, the posterior mid-ventral first pedicel, and the second pedicel, developed to the left from the mid-ventral radial canal. $\times$ 55.

Fig. 8. Ventral view of a larva of thirty-three days, in which the five primary tentacles, the posterior pedicel, and the second, third, and fourth pedicels, to the left from the mid-ventral radial canal, are prominent. The first pedicels from the right and left ventral radial canals have appeared. $\times$ 29.

Fig. 9. Dorsal view of embryo represented in Fig. 8, showing the three pairs of dorsal papillæ. The first two, at the anterior end of the body, are relatively large. The anus is guarded by two lateral, and one posterior, valves. $\times$ 29.

Fig. 10. View from the right side of a larva of forty days, showing five primary tentacles, the posterior pedicel, four well-developed pedicels to the left from the mid-ventral radial canal, and the three dorsal papillæ. In addition, the sixth tentacle, and the first pedicel to the right from the mid-ventral radial canal are now developed. $\times$ 29.

CHARLES LINCOLN EDWARDS.

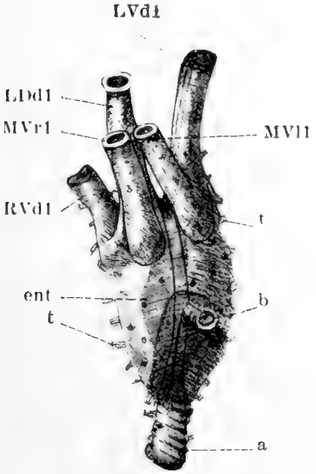

FIG. 7.
9 Days.

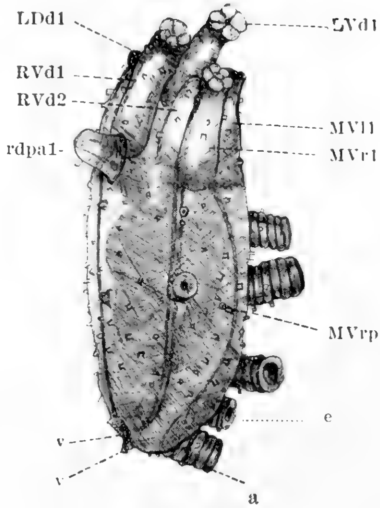

FIG. 10.
40 Days.

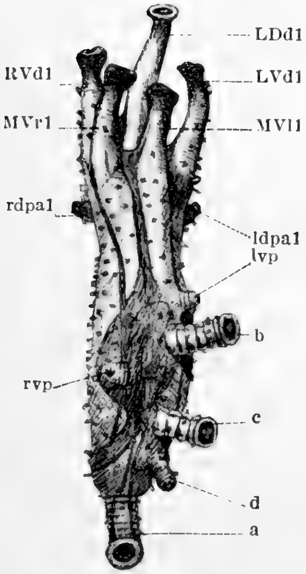

FIG. 8.

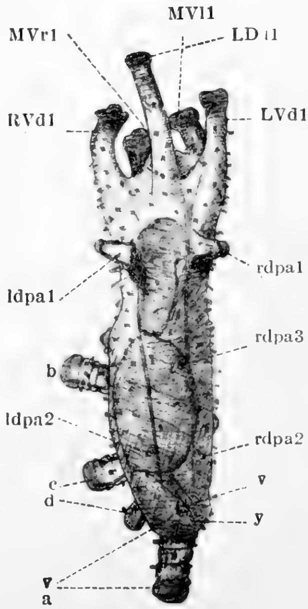

FIG. 9.

33 Days.

PLATE III.

Figs. 11, 12, 13. 14, show increased numbers of tentacles, pedicels, and papillæ. The terminal branches of the tentacles are well marked.

Fig. 11. Ventral view of larva of fifty-three days. In addition to the five primary tentacles, the sixth, seventh, eighth, ninth, and tenth, tentacles are shown. Four pedicels and three buds are now found on each side of the mid-ventral radial canal, forming a more or less zigzag line. Five developed pedicels and one bud arise ventrad from each lateral ventral radial canal. $\times$ 17½.

Fig. 12. Dorsal view of larva represented in Fig. 11. The two posterior pairs of dorsal papillæ have grown larger. An anterior pair of papillæ is developed dorsad from the right and left dorsal radial canals. $\times$ 17½.

Fig. 13. Ventral view of larva of seventy-five days, showing the additional thirteenth tentacle (LDd 2) and only a slight increase in the number of pedicels and buds over the fifty-three day holothurid. The mouth is well shown. $\times$ 14½.

Fig. 14. Dorsal view of larva represented in Fig. 13. The anus and anal plates are prominent. $\times$ 14½.

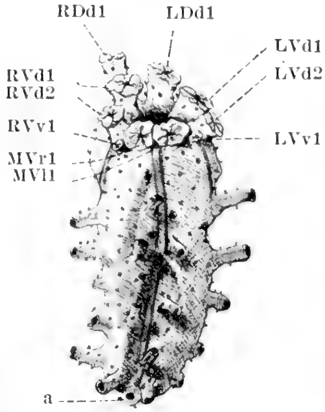

FIG. 11.

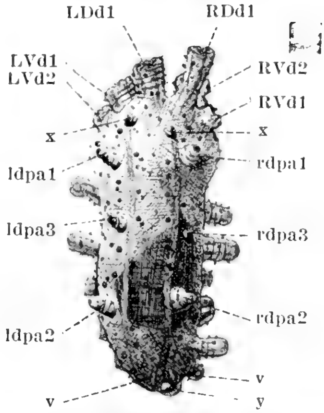

FIG. 12.

53 Days.

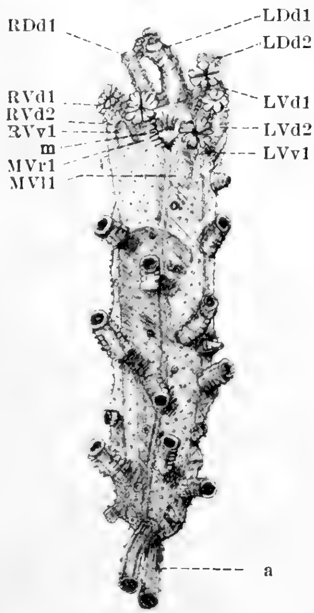

FIG. 13.

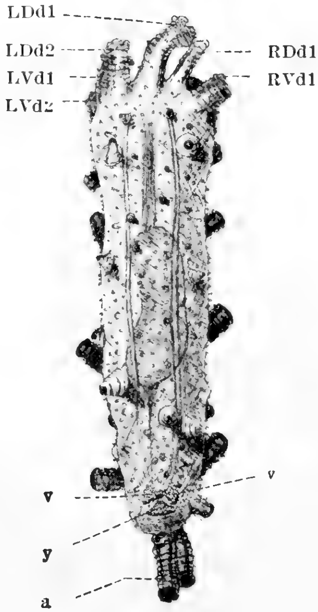

FIG. 14.

75 Days.

THE ORIGIN AND EARLY HISTORY OF THE GERM-CELLS IN SOME CHRYSOMELID BEETLES.

BY

ROBERT WILHELM HEGNER.

WITH 4 PLATES.

CONTENTS

I. INTRODUCTION.

The germ-cells of the Metozoa have been in recent years a favorite subject for investigators. The theory of the continuity of the germ-plasm expressed by Galton in 1872 and later (1885) elaborated

by Weismann has focused the attention of embryologists upon the reproductive cells. Many remarkable discoveries have been made in the late stages of the history of these cells, but comparatively little effort has been directed toward their early embryonic development. The work which forms the basis of the present paper was undertaken in an attempt to clear up some of the problems which have resulted from a large number of disconnected studies on the embryology of the Insecta. The lineage of the germ-cells of *Calligrapha multipunctata* is described in the following pages from a preblastodermic stage until the sex of the embryo can be recognized. Two other Chrysomelid beetles, *Calligrapha lunata* and *Leptinotarsa decemlineata,* are referred to in the course of the paper, but *C. multipunctata* has received the largest share of attention.

The work was begun at the University of Chicago and was continued at the Marine Biological Laboratory at Woods Holl, Mass., and at the University of Wisconsin. I wish to thank the members of the zoölogical staffs of these institutions for their kindness and for the facilities granted to me. I am greatly indebted to Professor Wm. S. Marshall for his valuable and generous aid and the use of his extensive library; and I am also under obligations to Professor C. O. Whitman for his helpful suggestions and advice. I wish to express my gratitude to my wife for her assistance in cutting sections and in preparing the manuscript for the publishers.

II. Review of the Literature upon the Origin of the Germ-Cells in the Insecta.

Introduction.

Only a few papers have been devoted exclusively to the origin and early development of the germ-cells of the Insecta. These contributions do not represent by any means our knowledge of this subject, for almost every work on general insect embryology contains observations on the primitive germ-cells, although usually in a subordinate degree.

Brandt (1878), Heymons (1891), Graber (1891) and others have provided a more or less complete historical account of this phase

of embryology, but the great number of recent papers dealing with the subject makes it advisable to give the following descriptions which attempt to present, in a brief manner, the numerous fragmentary sketches of the various authors.

1. *Lepidoptera.*

The earliest worker whose results are worthy of consideration is Herold (1815). He has given a remarkably good description of the gross aspects of both ovaries and testes in several species of Lepidoptera, principally *Papilio brassica,* from a late embryonic period to the adult stage. For us the chief value of his results lies in his discovery that the sex of the larva is already determined before hatching. He found the ovary to consist of four tubules, and the testis of four small sacs; the former with a duct at its posterior end and the latter with a duct extending from the center of its side.

In *Bombyx pini,* according to Heymons (1891), Suckow (1828), distinguished the male from the female before the larva hatched, thus confirming Herold's results. This author described the rudiment of the germ-glands as an outgrowth from the hind-intestine "das sich späterhin durch eine Furche theilt und nach und nach vom Darmkanale abgestossen als zwei seitlich verlaufende hohle Fädchen die Geschlechtsorgane im ersten Entwurf darstellt."

The reproductive organs could not be found by Meyer (1849) in *Liparis auriflua* until the caterpillars were over three weeks old, and he wrongly pronounced the young larvæ sexless. He was the first, however, to make finer histological examinations of the developing germ-glands.

Bessels (1867) made a more accurate microscopical examination of the embryonic germ-cells than did Meyer (1849), and, contrary to the results of the latter, he found that "Die Anlage der Sexualdrüsen findet bei den Lepidopteren im Ei statt, und es wird bereits hier die Verschiedenheit des Geschlechts vollkommen deutlich." In a late embryonic stage of *Zeuzera æsculi* he found the rudimentary germ-glands at either side in the eighth abdominal segment embedded

in the fat-body. They consisted of many transparent nucleated cells which separated to form the testicular follicles or ovarian tubules, about which a structureless membrane formed.

In *Tinea crinella,* Balbiani (1869-72) found that "l'organe sexuel est dèjà parfaitement perceptible à une époque oú l'embryon n'est encore représenté que par son rudiment ventral, et n'offre encore aucune trace de ses autres appareils organiques. A cette phase peu avancée de son existence, l'organe reproducteur forme une petite masse ovalaire simple, composée de minimes cellules rondes et transparentes. . . Cette masse est appliquée à la face interne de l'extremité inferieure du rudiment ventral. . . " This unpaired oval mass later divided, one-half going to either side of the body.

Brandt (1878) did not study the early development of the germ-cells but described both ovaries and testes in embryos of *Pieris brassica* just before hatching. "Die Anlagen der Genitaldrüsen selbst fanden sich im Embryo an der Rückenwandung der Leibeshöhle, etwa im achten Körpersegmente, rechts und links dicht am Herzen, dessen Peritonealüberzug auf sie überging. In ihrem jüngsten Entwicklungsstadium stellten sie je einen mehr oder weniger elliptischen Körper dar, welcher durchweg aus rundlichen Embryonalzellen mit amöboid gestalteten Kernen bestand." He found, as did Herold (1815), that the female germ-gland could be distinguished from the male by its duct which extended from the posterior end, while in the latter the duct is attached to the side.

O. and R. Hertwig (1881) found the rudiments of the germ-glands of *Zygæna minos* lying between the somatic and splanchnic layers of the mesoderm (Taf. II, Fig. 4).

According to Graber (1891), both Tichomiroff (1882) and Selvatico (1882) identified the germ-glands of *Bombyx mori* in comparatively early embryonic stages. The earliest embryo examined contained a germ-gland composed of a group of cells surrounded by mesoderm. It lay between the "Proctodæum und dem Mesenteron" in close connection "mit dem Faserblatt des Mitteldarmes."

The male germ-glands of *Zygæna filipendulæ* and other Lepidoptera were found by Spichard (1886) to arise from the mesoderm and

not from a muscle fiber (Schneider, 1885). In late embryonic life each germ-gland consisted of four large "Urzellen der Geschlechtsanlage" with small cells lying between them, the whole mass being surrounded by a flat-celled epithelium. As the four "Urzellen" increased by mitosis, the surrounding sheath grew inward, thus establishing the four testicular follicles.

The earliest appearance of the primitive germ-glands of Lepidoptera was recorded by Woodworth (1889) in *Euvanessa antiopa.* About the time the blastoderm was completed, a group of cells became cut off from the ventral plate near its posterior end; these cells remained "in contact with the ventral plate at the place where they are produced. Later stages show that these cells produce the generative organs. The generative organs thus appear to be produced by an infolding of the ectoderm, or possibly of the blastoderm before the ectoderm is produced but from a position which is later to become ectoderm."

In a nine and a half day embryo of *Pieris cratœgi,* Graber (1891) figured the "Anlagen der Samendrüsen" on either side of and dorsal to the intestine, thus occupying a position similar to that found by Selvatico (1882) in *Bombyx mori.* Each "Anlage" consisted of a few large cells and was surrounded by a small celled epithelium, the whole being embedded in fat-body.

Silk worm embryos, having appendages well developed, were found by Toyama (1902) to contain the rudiments of germ-glands. "Although the clusters of germ-cells are normally seen to occur in the third and sixth abdominal segments, we often observed them in all other abdominal segments with the exception of the anal; and in one case, we observed them even in the mesothoracic segment. We are thus in a position to say that the genital cells originally arise in each body segment." They differentiated from the cells of the mesoblastic somites.

The most recent account of the origin of the germ-cells in Lepidoptera was published by Schwangart (1905). He found in *Endromis versicolora* the first indication of a germ-gland two to four hours after blastoderm formation. A part of the blastoderm in the posterior region of the egg, but not at the posterior end, became

several layers thick; here the inner cells were large, richer in yolk, and their nuclei had one or two nucleoli, but less chromatin than the (overlying) blastoderm cells. These inner, primitive germ-cells soon became amœboid, and, several hours before mesoderm formation, separated into groups which moved forward through the yolk. Each group divided, half of the cells migrating to either side of the body, where they lay near the cœlom in the fourth to sixth abdominal segments. Their history was not carried further.

2. *Diptera.*

The Diptera have received their full share of attention from insect embryologists and the works relating to species of this order are very numerous. They are also the first, and for a long time the only insects in whose eggs pole-cells were discovered; this group is, therefore, of great interest to us since pole-cells are also present in the development of the Chrysomelid beetles considered in this paper.

Robin (1862) unknowingly discovered the early segregation of germ-cells from somatic cells in the nearly transparent eggs of *Tipulides culiciformes.* Before the blastoderm was formed there appeared "par gemmation de la substance hyaline du vitellus" a number of "globules polaires" which he likened to the polar bodies of other animals. These four to eight buds each developed a nucleus which gave to it the character of a true cell. These cells were supposed to take part in the formation of the blastoderm near the spot where they were protruded. Robin did not orient the eggs correctly for he says "C'est aussi le point où apparaitre plus tard l'extremité céphalique," a statement that Weismann corrected the following year.

Although Weismann (1863) had observed these "globules polaires" in the eggs of *Chironomus nigro-viridis* and *Musca vomitoria* several years before Robin's papers appeared, he did not publish his results until 1863. Weismann noticed at the pointed posterior end of the egg four indefinite, bright spots lying in the "Keimhautblastem." These developed into four bud-like protrusions which were entirely cut off from the egg and lay in the space between the vitelline membrane and the surface of the egg. These "globules polaires" or "Pol-

zellen," as Weismann called them, consisted of homogeneous protoplasm containing a nucleus and one or two yolk-granules. Weismann thought "dass der Modus ihrer Genese innerhalb des Begriffes von der freien Zellenbildung fällt, wie ihn die ältere histologische Schule aufgestellt hat." The four primitive pole-cells divided each into two, sometimes even before they were entirely separated from the egg; these resultant eight pole-cells became confused with the developing blastoderm-cells, and Weismann could not follow them further.

Neither Robin nor Weismann realized the importance of these pole-cells and it remained for Metschnikoff (1865) and Leuckart (1865) to announce their true significance. Leuckart (1865) described their formation in the pædogenetic larva of *Miastor* and confirmed Metschnikoff's (1865) statement that they were really the primitive germ-cells and entered into the constitution of the pseudovarium. Leuckart could distinguish the single nucleus of the primitive pole-cell when there were only eight to ten nuclei in the pseudovum.

A year later Metschnikoff (1866) gave a more detailed account of the history of the pole-cells in *Simula sp.* and *Miastor.* In *Simula* four to five pole-cells were present at the posterior end of the egg, each containing a nucleus and very fine yolk-granules. In *Miastor* the genesis of the pole-cells was traced more accurately than in *Simula.* Eggs were found containing only two nuclei which were supposed to result from the division of the germinal vesicle. These nuclei continued to give rise to others by division until twelve to fifteen were produced, one of which, lying at the pointed pole of the pseudovum, became surrounded by a thick, dark yolk mass and with it separated as a distinct membraneless cell, the first pole-cell. This then divided into two and later into four cells. These four then separated into two groups of two cells each and were recognized as the primitive reproductive organs lying in their definite positions. The two large pole-cells of each group were already divided and enclosed in an epithelial layer of embryonic cells at the time of hatching.

Grimm (1870) described in the parthenogenetic eggs of a species of *Chironomus,* a membraneless cell which divided into two, then

four pole-cells. He recorded this interesting variation. "Manchmal aber theilt sich der Kern der ersten Polzelle noch in der Schicht des Bildungsdotters liegend, so dass in dem Polraume auf einmal zwei Polzellen erscheinen." The division of these pole cells into two groups, each of which became surrounded by embryonic cells and constituted the germ-glands, confirmed Metschnikoff's (1886) account.

According to Packard (1872) the eggs of *Pulex canis,* one or two hours after deposition, contained "four distinct polar cells apparently immersed in protoplasm, and a small indistinct one in addition." They were distinctly nucleated and held in place by the vitelline membrane. Thirty hours later the blastoderm was fully formed and "Soon after this the polar cells break down and disappear."

In another flea, *Pulex felis,* Balbiani (1875) found that the "Anlage" of the reproductive organs was already visible after the formation of the embryonic envelopes. It was a small naked mass of clear cells lying in the posterior inner side of the abdomen.

In a later work Weismann (1882) described events in the pole-cell development of *Chironomus sp.* which differed from those found in *C. nigro-viridis* in 1863. In *Chironomus sp.* the primitive pole-cell nucleus was thought to have probably originated from elements of the cleavage nucleus in the yolk mass and migrated to the surface of the egg; there it entered a prótoplasmic process of the "Keimhautblastem" together with a number of yolk-granules which flowed in with it. This nucleus often divided, as Grimm (1870) had previously recorded, before the protrusion was entirely cut off from the egg, resulting in two pole-cells. A second bud arose in the same place as the first and passed through a similar process of separation and division. Thus four cells were produced; these increased by division to eight, and finally to twelve, which lay on the surface of the posterior pole. They did not become indistinguishable from the blastoderm-cells, as was described for *Chironomus nigro-viridis,* but "später wenn sich die Keimhaut gebildet hat, lagern sie sich deren Oberfläche locker auf, oft zu zwei Gruppen formirt." After the germ-band was formed two to four similar cells were found in many eggs outside of the embryonic membrane, but their derivation from pole-cells could not be determined.

Weismann described in one egg of *Chironomus sp.* a nucleated protrusion at the anterior end which was capable of amœboid movements and probably migrated upon the surface and was lost to view. In another case no nucleus was visible, but the anterior ball of protoplasm divided into eight bodies of different sizes, which after fifteen minutes dissolved. These were thought to be polar bodies, but might easily have been mistaken for pole-cells.

The pole-cells were accurately traced to the definitive germ-glands by Balbiani (1882, 1885) in *Chironomus.* One pole-cell usually appeared first; but, as previously observed by Grimm (1870) and Weismann (1882), sometimes two were protruded simultaneously. These two divided to form four and then eight pole-cells. Small non-nucleated protoplasmic globules were pushed out of the egg at both anterior and posterior ends, disintegrating later into a mass of granules in the polar cavities. Balbiani claimed that Robin (1862) and Weismann (1863), both of whom recorded twelve or more pole-cells, were deceived by these globules, as there should have been only eight pole-cells. These droplets would also explain the protrusion discovered by Weismann (1882) at the anterior end of the egg. Balbiani, however, did not find the anterior nucleated globules which Weismann considered polar bodies. He agreed with previous workers that the pole-cells did not arise by free formation during or after germination (Robin, 1862, Weismann, 1863), but were derivatives of the cleavage nucleus (Leuckart, 1865, Metschnikoff, 1866, Grimm, 1870). After the first division of the segmentation nucleus the anterior daughter nucleus probably gave rise to the blastodermic nuclei of that region, while the posterior furnished the two pole-cell nuclei and those of the posterior blastoderm-cells. After the eight pole-cells were formed, an elongation of the vitellus forced the group against the egg membrane causing a blastodermic depression.

In the next stage the pole-cells were found inside of the blastoderm, but Balbiani did not determine whether they passed through it, or a clear space was left in the blastoderm for their entrance. They now consisted of two separate masses, each containing two quadrinucleated cells. This was supposed to have come about in the following manner. The eight original pole-cells fused in pairs,

producing four binucleated cells; then the nuclei in these four cells each divided producing four quadrinucleated cells, two of which then moved to either side of the body. No further change took place within the two germ-glands until the time of hatching. Their position was altered by the contraction of the ventral plate and, in the young larva, they lay in the ninth abdominal segment level with the union of the mid- with the hind-intestine. Each germ-gland now acquired an epithelium of flattened cells. The testes could, at the end of embryonic life, be distinguished by their narrowness, attenuated ends, and many celled constitution, from the ovary, which was broad, obtuse, and composed of only a few large cells. One of Balbiani's conclusions was that "les glandes génitales des deux sexes ont une origine absolument identique, naissant de la même substance et au même point de l'oeuf."

Chironomus was also studied by Jaworowski (1882). This author was led to believe that the germ-glands of insects developed from a single mother cell, which gave rise by division to daughter cells, these in turn became mother cells and produced daughter cells. In this way the "Kammern," "Endfaden" and "Ausführungsgang" were all supposed to have arisen.

Three papers record the results of Schneider's (1885) observations "über die Anlage der Geschlechtsorgane der Insecten." Although his work was done chiefly on *Chironomus plumosus, C. plumicornis,* and a viviparous Cecidomyian, nevertheless he has made the following unique generalization: "Die erste Anlage der Geschlechtsorgane der Insecten besteht, soweit ich dieselbe verfolgt habe, in einer Muskelfaser, welche sich von einem Flügelmuskel abzweigt. Sie sitzt also vorn und hinten an der Hypodermis. In der Mitte derselben entsteht eine Anhäufung von Kernen durch welche die Muskelfaser erst spindelförmig, dann eiförmig aufschwillt. Wir wollen sie als die Geschlechtsanlage bezeichnen."

Kowalevsky (1886) observed in *Musca* that the division of the cleavage nucleus into two took place near the pointed end of the egg. The nuclei produced by these two reached the posterior end first and formed pole-cells which entered the cavity between the egg and the yolk membrane. The further history of these cells was not followed.

Voeltzkow (1889) stated that in *Musca* the pole-cells pressed the blastoderm-cells inward forming a wedge which projected into the yolk. Blastoderm-cells separated from this wedge, wandered into the interior, and became the so-called yolk-nuclei. He further stated that "die Polzellen wandern mit dem Keimstreifen auf die Dorsalseite und in den Enddarm hinein."

In his work on *Calliphora,* Graber (1889) described the pole-cells, but not until the blastoderm had formed. They were found outside of the blastoderm-cells and were smaller than the latter, and stained more intensely. "Ihre Zahl ist eine ziemlich constante und beträgt ca 25-35." They were figured in a cross section (Taf. VII, Fig. 91) where they occurred in the amniotic cavity. Graber seldom saw them in *Calliphora* and never observed them in *Lucilia.*

The section method was used by Ritter (1890) in studying the development of the germ-glands in *Chironomus.* He found that the first pole-cell differentiated at the posterior end of the egg when there were a large number of nuclei scattered about in the yolk. A second pole-cell was protruded close behind the first. Each carried out of the egg part of a flat mass of protoplasmic granules, the "Keimwulst," which, in section, formed a wreath around the nucleus. The two original pole-cells increased by division to four and then to eight. Two divisions of each pole-cell nucleus now occurred, resulting in eight quadrinucleated cells; these seemed to move of their own accord through the blastoderm which closed after them. They now lay at the posterior end of the germ-band from whence they were possibly moved anteriorly by the growing forward of the entomesoderm. The mass of pole-cells finally divided into two groups which occupied a position on either side of and dorsal to the hind-intestine; there they remained until after the larva hatched, when they became the definitive sex-organs. A more detailed account of Ritter's results will be found in Part IV on the pole-disc.

The germ-glands were found by Pratt (1893) in *Melophagus ovinus* during the entire larval period. They were small paired structures lying on either side of the abdomen embedded in the fat-body.

Lowne (1890-95) did not find the pole-cells in the Blow-fly,

probably, because of their small size, and similar appearance to other embryonic cells. In a 1 cm. larva he described as rudimentary germ-glands "two pairs of encapsulated groups of small embryonic cells, situated in the fifth abdominal segment embedded in the fat-bodies on either side of, and dorsal to, the alimentary canal." The pole-cells were considered by Lowne as "the segmentation spheres of the vegetative pole of the egg."

Escherich (1900) began his study of the embryonic development of *Musca vomitoria* and *Lucilia cæsar* after the formation of the blastoderm. He traced the history of the pole-cells from this time until the germ layers were completely separated. The pole-cells were first discovered as a group lying in a posterior enlargement of the ventral groove. From here they were carried around upon the dorsal side by the growth of the "Mittelplatte." The pole-cells migrated from here into the embryo through a "Polzellencanal;" they were last observed in the lumen of the "hinterer Entodermkeim" which was connected with the inner end of the proctodeal cavity.

Lecaillon (1900) has made the following general statement concerning the origin of the germ-cells in *Culex pipiens.* "L'étude du développement embryonnaire des insectes montre que, chez ces animaux, les cellules sexuelles, les *gonades,* se séparent de très bonne heure des cellules somatiques et semblent être toujours de nature ectodermique. Elles se groupent bientôt en deux petites masses pleines s'entourant chacune d'une enveloppe formée de cellules mésodermique aplaties. Souvent ces deux petites masses ne se modifient plus, au moins dans leur structure intime, et restent telles quelles jusqu' à la fin du developpement embryonnaire; ce sont les rudiments ou ebauches des organes génitaux. . . Pendant toute la durée de la vie larvaire, les organes génitaux demeurent à l'etat d'ébauche. Celle-ci consiste en deux petits massifs cellulaires situés dans la sixième anneau abdominal."

The most recent and best contribution to the genesis of the pole-cells of Diptera is that of Noack (1901). He found a dark granulated layer "Dotterplatte" at the posterior end of the egg of *Calliphora erythrocephala* similar to that discovered by Ritter (1890) in *Chironomus.* Each pole-cell took part of this layer of granules

with it as it passed through the "Keimhautblastem." This was the first cell differentiation. At this time there were fifteen to twenty pole-cells present, and not one to twelve, as Weismann (1863) and others have claimed; these pole-cells divided forming a single layer. This layer became several cells thick and formed the polar mass characteristic of Dipterous eggs at this stage of development. This mass of pole-cells was then passively carried into the dorsal groove of the germ-band where it was connected with the tissue destined to become the mid-intestine as Escherich (1900) had described it in *Musca vomitoria.* The pole-cells always remained connected with the yolk; they later began to wander through the entoderm, not by means of a definite canal (Escherich, 1900), but through an indefinite gap. After this migration had taken place, the pole-cells were found lying isolated among the entoderm cells from which they were distinguished by their darker pigmentation and smaller size. Soon, however, these distinguishing features became obliterated and the further history of the pole-cells could not be followed.

3. *Hemiptera.*

Until recently the Hemiptera have held a position next to the Diptera regarding the early appearance of germ-cells. As we shall see later germ-cells have been found by modern methods of study at a much earlier stage in the Hymenoptera and Coleoptera than thus far reported in the Hemiptera.

Huxley (1858) was the first to study the development of the pseudovarium in *Aphis.* He thought that the reproductive organs arose from the inner layer of the two-layered blastoderm.

Metschnikoff (1866) found in the viviparous Aphid, *Aphis rosæ,* shortly after blastoderm formation, a group of cells, "Keimhügel," projecting into the central yolk mass at the posterior end of the egg. These cells could not be distinguished from those constituting the blastoderm. The anterior part of this "Keimhügel" separated from the remainder and became an oval mass, the rudiment of the reproductive organs, "Genitalhügel." This rudiment, which lay within the tail fold, was carried by the latter into the position of the definitive germ-glands. Meanwhile, the nuclei increased in number, the

whole mass divided into horseshoe shaped halves which migrated to either side of the body. No "Genitalhügel" was found in *Aspidiotus nerii, Corixa* and *Psylla.* In the larva of *Psylla,* however, Metschnikoff described the germ-glands as follows: "Dicht neben den Lappen des secundären Dotters befinden sich jederseits bei den Larven und Imagines von *Psylla,* die Geschlechtsorgane—ein Umstand, welcher für meine Meinung über die Rolle des secundären Dotters als Fortpflanzungsmaterial zu sprechen scheint."

The development of both the parthenogenetic and the fertilized eggs of the Aphids was studied by Balbiani (1866-1872). In the eggs of the viviparous Aphids this author (1866) was able to see "un noyau granuleux fort pâle dans la vesicule postérieure, moins nettement dans l'antérieure, celle-ci présentent donc tous les caractères de veritables cellules. Ce sont ces vésicules ou ces cellules qui vont être l'origine des éléments générateurs mâles et femelles du futur animal, . . ." In the developing winter egg the first germ-cells were found by Balbiani (1869-1872) at a later stage. They formed an oval structure which lay in the median line of the body and soon divided lengthwise into halves.

Witlaczil (1884), in viviparous Aphids, also recorded a single primitive germ-cell which separated from the inner surface of the blastoderm at the posterior end of the egg. This single cell grew rapidly, producing by division a group of round clear cells each containing a large nucleus. This mass of cells was attached to the tail-fold, and moved with this until it reached a position in the posterior dorsal region of the body. During the revolution of the embryo, the rudiment of the germ-glands divided, half going to either side of the abdomen.

The origin of the germ-cells of viviparous Aphids was investigated by Will (1888). This author found that a thickening took place in the lateral wall of the blastoderm. "Bald aber sieht man die obersten Zellen der verdickten Cylinderseite sich in besonderer Weise differenziren; sie wachsen sehr schnell zu ansehnlicher Grösse heran, nehmen polyedrische Gestalt an und zeichnen sich durch eine geringere Tinctionsfähigkeit ihres Plasmaleibes sowie eine andre Beschaffenheit des Kernes aus. Durch rege Theilung vermehren sie

sich sehr lebhaft und stellen bald einen rundlichen Zellenhaufen, die Geschlechtsanlage, dar, welche in dieser Gestalt während der nächsten Entwicklungsstadien verharrt.

"Die Geschlechtszellen liegen auf der Grenze zwischen dem Entoderm, dem Ectoderm der Bauchplatte und dem gleich sich bildenden Mesoderm. Ueber ihre Zugehörigkeit zu einem der drei Keimblätter lässt sich streiten; mir genügt es vollkommen, zu constatiren, dass wir in ihnen indifferente Gebilde vor uns haben, die gerade dort entstehen, wo die drei Keimblätter an einander stossen."

4. *Hymenoptera.*

The Hymenoptera are represented in the literature of insect embryology by numerous papers chiefly on the bee. With the exception of the male of *Apis* (Petrunkewitsch, 1901) the germ-cells have not been traced back to as early a stage as in several other orders of insects.

Ganin (1869) was the first to investigate the germ-glands of the Hymenoptera. In *Platygaster* the rudiments of the germ-glands were described at the end of the first larval instar as separate thickenings on either side of the posterior end of the germ-band near the hind-intestine; between them arose a temporary unpaired elevation, "Geschlechtshügel," which disappeared during the second larval instar. In another Hymenopteron *Polynema,* Ganin found that the germ-glands developed from the undifferentiated cell mass of the tail-fold, and lay in the cavities of the last abdominal segments, where they remained during the larval period.

Bütschli (1870), in his work on the bee, described in a well developed embryo "nicht weit von den Rückenrändern der Leibeswandung jederseits eine durch ungefähr 5 Segmente sich erstreckende längliche Zellenmasse aus dicht gedrängten rundlichen mit grossen Kernen ausgestatteten Zellen bestehend," which he considered the rudiments of the germ-glands. Bütschli distinguished two layers in the germ-band of the bee, from the inner one of which, the entomesoderm, the primitive germ-cells were supposed to be derived.

Uljanin (1872), according to Brandt (1878), found in the larva

of the bee, two kidney-shaped bodies lying on either side of the dorsal vessel; he considered these to be the female sexual organs.

In several species of Hymenoptera Dohrn (1876) "fand in jungen Larven die Anlagen der Ovarien als einen breiten birnförmigen Körper, dessen breite Fläche in acht fingerförmige Fortsätze ausgezogen war, deren vier oben und vier darunter liegen."

Ayers (1883) studied *Teleas,* a parasite in the egg of *Oecanthus niveus.* Here the germ-cells were budded off from the dorsal side of a posterior enlargement of the nerve cord which curved upward around the end of the mesenteron. A varying number of cells (two to six) were thus produced embedded in homogeneous protoplasm. "They appear in sections of hardened specimens as though formed endogenously within the substance of the still persisting mother cells. . . These are shortly separated from the nervous cord, but are connected to the blind end of the mesenteron by protoplasmic filaments, usually one to each mother cell."

The embryonic germ-glands of the bee were described by Grassi (1884) as two solid strands, extending from the fourth to the eighth abdominal segment. "Sono formazione mesodermica; nascono press' a poco ai confini tra il foglietto superficiale e il foglietto profondo del mesoderma. . . ."

The rudiments of the germ-glands were found by Carriere (1890) in *Chalicodoma muraria* at about the time when the first pair of Malpighian tubules appeared. They lay near the dorsal wall of the "Urhöhle" in the fifth and sixth abdominal segments.

Bugnion (1891) studied the postembryonic development of *Encyrtus fuscicollis.* The rudiments of the reproductive organs were found in the middle of larval life; they were oval structures lying on either side of the hind-intestine. During the second half of larval life the sexes could be distinguished; the testis remained small and round, while the ovaries became oval and larger.

The "Anlage" of the germ-glands were found by Kulagin (1897) in the parasitic Hymenopteron, *Platygaster herrickii,* lying near the hind-intestine of an embryo in which the mesodermal somites were forming. This rudiment was a paired structure composed of cells similar to those of the mesentoderm, the only difference being their

tendency to stain more intensely. "Dafür spricht erstens die frühe Absonderung der Geschlechtszellen, schon zu einer Zeit, wo das Meso- und Entoderm nicht scharf getrennt ist, und ferner die sehr grosse Aehnlichkeit bei den Zellelementen in den ersten Stadien ihrer Entstehung."

According to Carriere and Bürger (1897) the primitive germ-cells of *Chalicodoma muraria* were probably derived from cells of the mesodermal layer shortly after its appearance. A few cells in the dorsal wall of the somites of the third, fourth and fifth abdominal segments on either side of the body proliferated to form egg-shaped bodies. In further growth these germ-cells decreased in size as a result of multiplication. Later, they wandered from the third and fourth segments into the fifth, where they lay behind one another in their original succession.

In an endeavor to test the "Dzierzon theory," that the eggs which produce drone bees are normally unfertilized, Petrunkewitsch (1901-03) discovered some unusual maturation divisions. In "drone eggs" the first polar body passed through an equatorial division, each of its daughter nuclei containing one-half of the somatic number of chromosomes. The inner one of these daughter nuclei fused with the second polar body, which also contained one-half of the somatic number of chromosomes; the resultant nucleus with sixteen chromosomes, the "Richtungscopulationskern" passed through three divisions giving rise to eight "doppelkernige Zellen." After the blastoderm was completed, the products of these eight cells were found in the middle line near the dorsal surface of the egg, where the formation of the amnion had already begun; the nuclei of these cells were small, and lay embedded in dark staining cytoplasm. Later they were found just beneath the dorsal surface near the point of union of the amnion with the head-fold of the embryonic rudiment. They were next discovered between the epithelium of the mid-intestine and the ectoderm; from here they migrated into the cœlomic cavities, and finally, at the time of hatching, formed a "wellenartigen" strand, the germ-gland, extending through the third, fourth, fifth and sixth abdominal segments. The fertilized eggs of the bee were also examined by Petrunkewitsch, but no "Richtungscopula-

tionskern" was present. In these eggs "entstehen die Genitaldrüsen aus Mesodermzellen, die in die Mesodermröhren von der Bauchseite hereindringen."

5. *Orthoptera.*

Many papers have appeared contributing to our knowledge of the germ-cells of the Orthoptera. Unfortunately, a large number of them are based on a study of one species, *Blatta germanica,* which is not favorable for this particular phase of embryological research.

We owe the first account of the primitive germ-cells of the Orthoptera to Ayers (1883). He found the rudiments of the germ-glands in *Oecanthus niveus* at a late embryonic stage after revolution of the embryo had taken place. "They are first seen as two irregular groups of amœboid cells, belonging to the splanchnic layer of the mesoderm on either side of the dorsal vessel." These groups of cells were transformed into ovaries in their primitive position.

Nusbaum (1886) considered the reproductive organs of *Periplaneta orientalis* of mesodermal origin.

Heymons (1890-91) made a detailed study of the origin and development of the germ-glands of *Blatta germanica.* He found in this insect a segmental origin of the germ-cells. They were identified as large cells, in the second to the seventh abdominal segments, which arose from the splanchnic layer of the mesoderm; new genital cells were continually added. Later a migration of the germ-cells took place into the cœlomic cavities, and afterward between the cells of the dorsal walls of the cœlomic sacs. Here a continuous strand of germ-cells was formed on either side of the body; these were situated in the dorsal wall of the primitive somites extending from the second into the eighth abdominal segment. Undifferentiated mesoderm cells were added to these strands from the dorsal wall of the cœlomic sacs, to form the epithelium of the germ-glands. The strands now shortened, and, by the lateral pushing of the germ-band around the yolk, were carried to the dorsal side of the egg where they continued their growth.

Heymons (1890) claimed to have discovered an hermaphroditic condition in the male genital organs of *Blatta* (*Phyllodromia*). "Es geht hieraus unzweifelhaft hervor, dass jener Theil der Genitalanlage

beim Männchen, welcher nicht mit zur Bildung der Hodenfollikel verbraucht wird, die Anlage zu einer weiblichen Genitaldrüse darstellt." Other workers have not sustained Heymon's interpretation.

Several papers on the embryology of *Blatta* (*Phyllodromia*) *germanica* were published by Cholodkovsky (1890-91). This author differed in some points from Heymons (1890-91). He found the primitive germ-cells always lying in the dorsal wall of the cœlomic cavities pre-eminently if not exclusively in the fifth and sixth abdominal segments. The number of germ-cells increased either by division or by the addition of new cells which penetrated into the cœlom. As the embryo grew dorsalward around the yolk, the germ-glands were carried to a point on either side of and dorsal to the mid-intestine. Cholodkovsky did not agree with Heymons that the germ-cells differentiated from mesoderm-cells, but held that they probably were derived from yolk-cells.

Stenobothrus variabilis was studied by Graber (1891). The first distinct rudiments of the germ-glands were found as two large faintly stained cells differentiated from the visceral layer of the dorsal "Mesoblastdivertikel." At the end of the embryonic period the sexual organs appeared as two strands lying close to one another on the dorsal side of the posterior region of the mid-intestine.

The germ-glands of *Mantis religiosa* seemed to Graber delayed in their development as compared with those of *Stenobothrus,* for, in the former, they still retained their lateral position at the end of embryonic life.

Wheeler's (1893) paper on *Xiphidium ensiferum* and other Orthoptera contains a short account of the development of the germ-glands. In the above named species the first sign of the primitive germ-cells was not discovered until the somites were established as distinct sacs. The germ-cells at this time lay in the splanchnic walls of the somites of the first to the sixth abdominal segments; one group was found in the tenth abdominal segment. They were larger and paler than the mesoderm-cells and were thought to have been derived from the latter. A cluster of cells was formed by the mitotic division of these primary germ-cells. The somites that bore germ-cells each sent out a solid diverticulum which connected with

the antecedent somite, thus producing a continuous strand. The hexametameric arrangement of the germ-cells disappeared as this strand shortened to form the ovary or testis. Wheeler noted the presence of germ-cells in the first abdominal segment of *Blatta germanica* in opposition to Heymon's statement that "Im ersten Abdominalsegment treten niemals Genitalzellen auf."

Heymons' earlier papers were supplemented by a re-examination (1895) of *Blatta* (*Phyllodromia*) *germanica* and a clear account of the development of other Orthoptera, *Periplaneta orientalis, Gryllus campestris, Gryllus domestica* and *Gryllotalpa vulgaris.*

In *Blatta* he was able to trace the germ-cells back to an earlier stage than recorded in his former paper (1891). When the posterior amniotic fold arose, a groove, "Geschlechtsgrube," appeared in the posterior end of the germ-band. Many cells became detached from the bottom of this groove and wandered into the interior; they moved singly and "sich teils zwischen den Mesodermzellen, teils über sie hinweg nach vorn bewegen." They assumed the character of the germ-cells only when they arrived at the visceral walls of the somites where they behaved just as previously stated (Heymons, 1891).

In *Periplaneta* a "Geschlechtsgrube" also appeared similar to that found in *Blatta;* cells separated from it and migrated by amœboid movements toward the anterior abdominal segments where they arranged themselves intersegmentally forming wedge-shaped accumulations between the cœlomic sacs. Contrary to the condition in *Blatta,* these could be distinguished as germ-cells shortly after they became detached from the ectoderm. Later these germ-cells separated to form two strands lying on either side of the body in the visceral walls of the second to the seventh abdominal segments; they acquired an epithelial layer derived from mesoderm-cells.

A "Geschlechtsgrube" was found by Heymons (1895) in both *Gryllus campestris* and *G. domesticus,* and the germ-cells arose in a manner similar to that in the Orthoptera previously described. These two species differed only in the fact that the germ-cells differentiated earlier in the former than in the latter. A long oval structure was produced by the accumulation of the primitive germ-

cells; this body divided during the segmentation of the germ-band, one half going to either side, where it lay in the dorsal region of the second and third abdominal segments. Here the germ-gland was enclosed in an epithelium of mesoderm-cells.

Gryllotalpa vulgaris was not so thoroughly studied as the other species. Heymons, in this Orthopteron, found "nur sehr flache Einstülpung. Von dem Boden der Letzteren lösen sich Zellen los und wandern ein. Ich bin geneigt, diese Zellen als Geschlechtszellen anzusehen, obwohl sie das Aussehen von Mesodermzellen haben und ich ihr weiteres Schicksal nicht verfolgt habe."

6. *Coleoptera.*

The Coleoptera are represented in the literature on the embryology of insects by only a few papers; of these eight contain fragmentary accounts of the origin and early development of the germ-glands.

Heider (1889) found that in *Hydrophilus piceus* the germ-glands originated from the inner wall of the primitive abdominal segments on either side of the body. They arose as solid outgrowths from that part of the wall of the somites which lay between the place of origin of the Fettkörperband" and the splanchnic layer of the mesoderm.

The germ-glands of *Leptinotarsa* (*Doryphora*) *decemlineata* were described by Wheeler (1889) as follows: "These organs originate as two elongated thickenings of splanchnic mesoderm, one on each side projecting into the body cavity. Later they become rounded and are attached by a thin band of splanchnic mesoderm only." Wheeler found several cells in the posterior amniotic cavity of an embryo of *Leptinotarsa* which he concluded might be comparable to the pole-cells of Diptera. The origin and fate of these cells was not determined.

In *Melolontha vulgaris,* the sexual organs were found by Voeltzkow, (1889) "zu der Zeit, wo die Darmwülste sich am Bauch zu schliessen beginnen und die Leibeshöhle sich fertig gebildet hat. Sie werden vom Mesoderm aus gebildet und liegen am hinteren Ende des Eies als ein Paar birnförmige Gebilde, umgeben von einer starken, ringförmigen Zellenmasse mit grossen Kernen. . . . Später rücken

sie etwas nach dem Rücken hinauf und liegen rechts und links vom Rückengefäss."

Three species of beetles, *Hydrophilus piceus, Melolontha vulgaris* and *Lina tremulæ* received brief mention by Graber (1891). In *Hydrophilus* the two germ-glands were found lying near each other on the dorsal wall of the intestine, close to the proctodeum. In *Melolontha* "Die Gonaden erscheinen hier sehr frühzeitig als mit dem Visceralblatt verbundene gestielte Körper, an denen man wieder ein Zellepithel und mehrere grössere und kleinere Inhaltszellen unterscheiden kann." In *Lina* the reproductive organs were discovered anterior to the proctodeum in connection with the "Darmfaserblatt." They appeared very similar to the corresponding organs described by Wheeler (1889) in *Leptinotarsa.*

In a Russian paper, Nusbaum (1891) has figured at the posterior pole of the egg of the oil beetle, *Melæ proscarabæus,* a wedge-shaped structure designated as an "accumulatio plasmatis et nucleorum in posteriore polo ovi." This nucleated mass occupied the position of, and is very similar in appearance to, the group of pole-cells and pseudoblastodermic nuclei shown in Fig. 25; in this species there probably occurred an early development of the germ-cells, such as has been found in Chrysomelid beetles.

The embryological development of the following species of Chrysomelidæ was studied by Lecaillon (1898); *Clytra læviuscula, Gastrophysa raphani, Chrysomela menthastri, Lina populi, L. tremulæ Agelastica alni.* In *Clytra,* the principal form examined, Lecaillon found the first nuclei which arrived at the posterior pole of the egg to become the primitive germ-cells; these could be distinguished from neighboring cells by their large size, larger nuclei, and darker cytoplasm. The germ-cell nuclei did not stop when they reached the surface of the egg, but passed outside and became separated from it; their number increased "peu à peu par suite de l'arrivée de nouvelles cellules périphériques et aussi sans doute de la division des premières cellules détachées du pôle de l'œuf." The germ-cells then started to re-enter the egg, retarding, by this migration, the formation of the blastoderm at this point. "Finalement, le blastoderme achève de se former au pôle posterieur de l'œuf, et alors

les cellules sexuelles se trouvent groupées . . . entre le vitellus et l'enveloppe blastodermique." At the end of segmentation, the germ-cells were found pressed against the inner surface of the germ-band, just in front of the posterior end of the egg; here they remained during the formation of the mesoderm. After the mesodermal somites were completed, the germ-cells penetrated into them, and formed two cylindrical groups, the germ-glands. These were then carried by the lateral growth of the embryo to a point near the median dorsal line.

The above described processes also took place in *Chrysomela menthastri, Lina populi* and *L. tremulæ.* The primitive germ-cells of *Gastrophysa* arose as in *Clytra,* but many of them remained outside of the egg and were later found in the posterior amniotic cavity, "dans le sillon profond qui se trouve sur le milieu de sa paroi interne. Avant la fermeture du sillon, les cellules y pénétrant et ses trouvent ensuite en dedans de la couche ectodermique." No observations were made by Lecaillon upon the precocious appearance of the germ-cells in *Agelastica alni.*

Several species of Chrysomelid beetles were studied by Friederichs (1906). This author discovered that the cleavage nuclei in *Donacia crassipes* reached the posterior later than the anterior end of the egg; the reverse is the rule in species of allied genera. After the blastoderm was formed "an der Ventralseite unmittelbar seitlich vor dem Pol, findet eine besonders lebhafte Zellvermehrung statt, so dass einzelne Zellen aus dem Blastodermverband heraus und ins Innere gedrängt werden." These, the primitive germ-cells, were not very different in *Donacia* from blastoderm-cells, but in *Timarcha nicæensis* and *Chrysomela marginata* they were distinguished by the larger size and darker color of their nuclei. The blastoderm (Ectoderm) became interrupted at the point of origin of the germ-cells, an invagination being found similar to the "Geschlechtsgrube" of Orthoptera (Heymons, 1895). The germ-cells remained just inside of this groove; by the lengthening of the embryonic rudiment they were carried to a point near the mid-dorsal region of the egg. Here they were found at the end of the tail-fold, lying between the ectoderm and the yolk. The germ-cells, as well as the other cells of the embryo,

gave off "Paracytoide" which helped dissolve the yolk. These "Paracytoide" arose either from degenerated nuclei or from "Kernzerlegung" and contained "keine Chromosomen, sondern nur Trophachromatin (Chromidialkerne)".

The most recent contribution to the development of the germ-glands of the Coleoptera is that of Saling (1907). This paper deals exclusively with the origin and growth of the reproductive organs of *Tenebrio molitor*. Saling did not find in this species such a precocious differentiation of germ-cells as Lecaillon (1898) recorded for Chrysomelid beetles, but says: "Dagegen halte ich für wahrscheinlich, dass ihre Loslösung vom Ectoderm am hinteren Keimstreifende erfolgt, sobald sich die hintere Amnionfalte erhebt und die Mesodermbildung im Gange ist. Beim Vorwärtsdringen der sich segmental anordnenden Mesodermmasse schiebt sich auch die noch unpaare Genitalanlage weiter nach vorn und gelangt vor Ausbildung der Ursegmente an die Grenze des sechsten und siebenten Abdominalsegments. Durch eine Teilung in lateraler Richtung wird sie paarig und tritt mit den inzwischen ausgebildeten Cölomsäcken des siebenten Abdominalsegments in Verbindung. Erst von diesem Zeitpunkt an ist die Genitalanlage bei *Tenebrio molitor* mit Sicherheit zu erkennen." The germ-cells penetrated through the median wall of the abdominal segments to the cœlomic cavities gathering a mesodermal epithelium during this migration. Sex-differentiation took place shortly before the end of the embryonic period.

7. *Neuroptera.*

The origin of the germ-glands in the Odonata and Ephemerida is still unknown. Heymons (1896) was unable to find the germ-glands in the embryos of the dragon-fly, but in the larva discovered them lying on either side of the intestine. They were spindle-shaped and composed of only a few cells. It was also impossible to find the germ-glands of *Ephermera* during embryonic life. Heymons concluded "dass bei den Odonaten und Ephemeriden die Differenzirung der Geschlechtsdrüsen nicht beim Embryo, sondern erst bei der Larve sich abspielt."

8. *Dermaptera.*

The primitive germ-cells were identified by Heymons (1895) in *Forficula auricularia* soon after the blastoderm was completed, forming a group at the posterior pole of the egg. At this time they could not be distinguished from the blastoderm-cells, but soon their nuclei became larger and clearer. The germ-cells increased rapidly by division and formed a spherical body, the genital rudiment. "Paracyten" were abundant near the germ-cells, and "Auch einzelne Genitalzellen pflegen nicht selten zu degeneriren, und zwar unter denselben Erscheinungen, die wir an den Paracyten kennen gelernt haben." The genital rudiment was pushed anteriorly near the dorsal surface of the body, by the lengthening of the germ-band. When the primitive segments appeared, the germ-cells, which lay in the tenth to the eleventh segments, were, by the bending of the posterior end of the body, forced into the ninth segment. They now separated from one another and migrated anteriorly by means of amœboid movements until they reached the sixth and seventh abdominal segments. Sometimes a few germ-cells were left behind in segments eight, nine, ten, or eleven. Now the germ-cells separated, half going to either side of the embryo, and moved anteriorly into the third to the seventh abdominal segments. Most of the germ-cells of the male remained in segments five, six and seven; those of the female were distributed approximately uniformly through segments three to seven.

9. *Aptera.*

Heymons (1897) has given a clear account of the primitive germ-cells in a *Thysanuran, Lepisma saccharina.* In this insect a knob-like projection was observed at the hinder end of the germ-band; this projection was composed of cells with large nuclei containing less chromatin than the nuclei of the mesoderm-cells, which had also begun to appear. These larger nuclei were interpreted as germ-cells and arose from the ectoderm. The primitive germ-cells migrated just as they were found to do in Orthoptera (Heymons, 1895), "Stets gelangen die Geschlechtszellen an die dorsalen Ursegmentwandungen, dringen in dieselben ein und bilden zusammen mit den

Mesodermzellen der letzteren die Genitalfollikel." A strand of cells was formed on either side of the body and from each, in the female, five pairs of follicles arose and were distributed in segmental order from the second to the sixth abdominal segment. In the male, double pairs of follicles occurred in the fourth to the sixth abdominal segments. There were thus produced twelve "Hodenfollikel" and ten egg-tubes.

Very little attention has been directed to the early development of the germ-glands in the Collembola, but several embryologists have, however, made observations on the later stages of these organs.

Claypole (1898) described two methods of origin of the germ-cells in *Anurida maritima.* In the first case a germ-cell was found lying in the cavity of the mesoblastic somite of the second abdominal segment; this gave rise to a mass of cells, the germ-gland, situated between the splanchnic and somatic layers of the mesoderm. Later, the germ-gland appeared to break through the mesoderm and come in contact with the yolk. The ovaries which developed in this way were found to contain many yolk globules lying among the germ-cells.

In the second case, Claypole states that a single germ-cell passed out from the wall of the mesoblastic somite into the yolk; it produced an irregular mass of cells lying partly in the mesoderm and partly in the yolk. The cells of this group migrated inward becoming scattered among the yolk-globules. The next period in the history of these cells was not found. The final stage found by this author represented the germ-gland of the male; it was an oblong structure directly connected with a large irregular sac of yolk.

The ovaries of ten species of Collembola were studied by Lecaillon (1901). In *Sira nigromaculata,* just after hatching, the female sex-organs were described as "deux petites masses ovoides placées dans la cavité générale du corps, à peu près a égale distance de l'extrémité antérieure et de l'extrémité postérieure de l'abdomen." They were situated ventrally between the third and fourth abdominal segments. The germ-glands of *Tomocerus plumbeus* and *Templetonia nitida* were found to occupy a position similar to that described above. In *Templetonia,* however, "les dimensions de la chambre gonadiale surpassent notablement celles du même organe chez les deux espèces précédentes."

III. Observations.

1. *The Pole Disc.*

A disc-shaped mass of dark staining granules is present at the posterior end of the freshly laid eggs of both *Calligrapha* and *Leptinotarsa;* these granules lie suspended in the inner stratum of the "Keimhautblastem." This layer of granules, which I shall call the pole-disc, plays one of the most important rôles in the genesis of the pole-cells; on this account I shall in this place describe its early characteristics, leaving its later history to the succeeding chapter.

Unfortunately, material was not at hand with which to trace the origin of this pole-disc, the earliest stage in my preparation (Fig. 2) being the eggs of *Leptinotarsa* taken from the oviduct just before deposition. At this time the pole-disc is present in the position it occupies throughout the entire course of the early cleavage of the egg. Under low magnification it appears in longitudinal sections as a dark irregular line lying just within the surface at the posterior end of the egg; under high powers, however, it is seen to be granular in structure. In the section figured (Fig. 2) it occupies the innermost portion of the "Keimhautblastem;" its granules are grouped together in small masses giving the entire pole-disc the appearance of a broken strand. These granules are easily distinguished from the cytoplasm in which they are suspended, by their large size and susceptibility to various stains; they appear to be arranged around small vacuoles which vary in size in the different preparations examined, irrespective of the age of the egg. Thus the granules in the pole-disc under consideration (Fig. 2) are crowded as closely together as they ever become, while in other eggs taken from the same batch they were widely separated.

Fig. 1 represents the posterior end of an egg of *Calligrapha;* here we see that the pole-disc occupies about one-eighth of the total area. The central part of the disc is denser than it is in any other region of it except the periphery, where an irregular margin is produced by numerous dark projections. Longitudinal sections of the pole-disc (Fig. 3) explain this difference in density, as it is found to be thickest in the center, and to be thrown into irregular folds at

the ends causing these portions to appear darker when seen in surface view.

The condition exhibited by most of the discs examined is represented in Fig. 3; the granules are not crowded closely together as in the disc described above (Fig. 2), but lie distinct from one another, forming an alveolar-like structure (a network in longitudinal section). Large vacuoles occur near the outer and inner surfaces of the disc, and in some cases yolk-globules come almost in contact with its upper side. Scattered about in the cytoplasm near the margin of the disc, are a number of its granules which have become separated from the main structure.

A third disc (Fig. 4) illustrates another condition which is not unusual, especially in the later cleavage stages of the egg. The granules have apparently lost their alveolar arrangement, now being diffused throughout a greater portion of the "Keimhautblastem." Within this disc are several large vacuoles surrounded by regular layers of granules.

The granules of the pole-disc are very susceptible to stains; in hæmatoxylin, thionin and gentian violet a color was obtained as deep as that of the chromatin in either dividing nuclei or those in a resting condition; they stained in orange G more intensely than the surrounding cytoplasm and almost as deeply as the yolk-globules. Other stains failed to bring out any further variations in the results.

2. *The Genesis of the Pole-Cells.*

Before describing the genesis of the pole-cells of *Calligrapha multipunctata* it seems desirable to give a brief account of the maturation, fertilization and early cleavage of the egg.

Eggs that have just been laid contain polar bodies in various phases of formation; these are given off into a thickening of the "Keimhautblastem" at a point slightly anterior to the median transverse axis of the egg. Here they remain and later disintegrate.

The female pronucleus lies in an amœboid accumulation of cytoplasm among the yolk-globules. It moves inward and conjugates with the male pronucleus at a point level with the polar bodies.

Here the first cleavage divisions take place. A number of the earliest cleavage stages were found (2, 4, 6, 16, etc.) in all of which the majority of the nuclei were nearer the anterior than the posterior pole. This anterior position of the early cleavage nuclei has already been described for Coleoptera in *Hydrophilus* (Heider, 1889), and in many insects belonging to other orders.

As cleavage progresses a separation of the nuclei into two sections takes place. The nuclei of one group form a more or less regular layer equidistant from the periphery at all points except the posterior end; here a space wider than elsewhere separates them from the surface of the egg. This layer is composed of preblastodermic nuclei (by nucleus is meant the nucleus plus its accompanying cytoplasm) which move outward, fuse with the "Keimhautblastem" and produce the blastoderm. The nuclei of the other group (vitellophags) remain behind scattered throughout the yolk.

When the preblastodermic nuclei have almost reached the peripheral layer of cytoplasm (Fig. 5) it is possible to distinguish those whose descendants will come in contact with the pole-disc from the neighboring nuclei which will produce ordinary blastoderm-cells. This distinction can be made more easily in a polar surface view (Fig. 12) which shows the entire pole-disc. Such a view discloses eight nuclei lying directly under the central area of dark granules. These nuclei, as we shall show later, divide twice before reaching the periphery of the egg. Some of the nuclei thus produced will, however, pass outside the margin of the disc and take part in blastoderm formation; the others, which will enter this granular area and become the pole-cells, can be traced back to the row of four nuclei which lie in the center of the pole-disc nearest the surface of the egg. At this stage (Fig. 5) all of the nuclei within the egg are similar, the various stains used (see the chapter on methods) failing to bring out any differences in structure. Differentiation takes place only when those nuclei in the posterior region fuse with the "Keimhautblastem." Continued division brings the preblastoderm-nuclei into the position shown in Fig. 6, where they again increase in number by mitosis. In the section figured (Fig. 6) all of the nuclei are in the prophases of mitosis, and each of those nearest the pole-disc (Fig. 6, *a*) has its

chromatin partially arranged in an equatorial plate. An enlarged view of the two nuclei which lie nearest the posterior pole (Fig. 18) shows that each is surrounded by an amœboid mass of cytoplasm with many fine pseudopodia-like processes extending outward in all directions, finally becoming lost among the yolk-globules. The nuclear membrane of each nucleus has disappeared, but its former position is clearly marked by the difference in density between the cytoplasm and the nuclear sap; a fusion of these two substances has not yet taken place. No nucleolus is visible.

After this division is completed, the daughter nuclei are closer together and nearer the surface of the egg (Fig. 7, *a*). The two nuclei with their accompanying cytoplasm, which now lie closest to the posterior pole (Fig. 19), have finally come in contact by means of their pseudopodia, with the "Keimhautblastem." These two nuclei are in a resting condition; their chromatin is evenly distributed throughout, the chromomeres lying in a linin reticulum singly or in groups of from two to six or more. One small nucleolus is present. All the other nuclei in the egg are similar in appearance at this time, and no differences in size or structure could be distinguished. It is not unusual to find all of the nuclei within the egg in repose at the same time, as mitosis is very rapid, and the subsequent resting period relatively long; thus, although nuclei in different regions are often found to be in different phases of division, nearly all of the sections made from eggs in preblastodermic stages show every nucleus in a resting condition. Each nucleus is in repose at this time (Fig. 19), but the cytoplasm accompanying it is still actively engaged in its migration toward the periphery. The cytoplasm surrounding the nuclei reaches the "Keimhautblastem" and the granular pole-disc simultaneously, the latter at this point occupying the inner portion of the former as already described in the preceding chapter.

Each nucleated mass of cytoplasm that comes within the limits of the pole-disc presses outward a mass of granules equal to the area of its fore-end. The whole disc is thus indented in as many places as there are protoplasmic masses striking it (Fig. 8 and Stage A). Fig. 20 illustrates two stages (*a* and *b*) in the early genesis of the pole-cells, and also shows at the extreme right a third nucleus (c)

which lies outside of the pole-disc and is destined to become a part of the blastoderm. The central nucleus of this trio (*b*) has pushed a part of the pole-disc outward and forced the granules into a cap (a half-moon in section) which extends half-way up its sides. The granules are now not as widely separated from one another as we found them in a previous stage (Fig. 3); they have become more densely packed due to the pressure exerted by the migrating pole-cell nucleus in its effort to break through the "Keimhautblastem" (compare Figs. 4, 18 and 19). The neighboring pole-cell nucleus (Fig. 20, *a*) is in a somewhat more advanced stage; it shows that the granules covering its outer surface become, in part, pushed away from this region and gradually extend around its sides until they cover all of the surface except the innermost portion. All of the granules of the pole-disc do not adhere to the pole-cells, some being left behind within the remains of the "Keimhautblastem."

A surface view of the posterior end of the egg at this stage shows plainly eight pairs of pole-cell nuclei (Fig. 13); an enclosing layer of the dark staining granules is suspended within each of these enabling one to distinguish easily the pole-cell nuclei from the adjacent blastoderm-nuclei. This paired condition of the pole-cell nuclei is accounted for by the fact that since the division of the eight nuclei of the previous generation, the daughter nuclei have not yet separated. In one case where the daughter nuclei have drawn some distance apart from each other (Fig. 13, *b*) a wide strand of cytoplasm is seen connecting the two. A few of the neighboring blastoderm nuclei, which have passed near the edge of the pole-disc, have also carried with them some of the granules which are seen embedded in their adjacent sides (Fig. 13, *a*). The nuclei of the blastoderm are slower than the pole-cell nuclei in reaching the surface, and have hardly begun to protrude when the latter have almost, or, in some cases, entirely separated from the egg (Fig. 9). Each pole-cell nucleus is now completely surrounded by a layer of granules more dense at the side than at either end (Fig. 21). After reaching the position last described (Fig. 9) all of the pole-cell nuclei, except in a few cases where one of them has been delayed (Figs. 26 and 22) become entirely separated from the egg, and lie in a single

layer between the remains of the "Keimhautblastem" and the vitelline membrane. They now vary from one another both in the size of the entire cell and in that of the nucleus. The long cytoplasmic processes which were characteristic of the pole-cell nuclei while within the egg have disappeared; they have undoubtedly been retracted until they now form a few blunt projections still suggestive of pseudopodia (Fig. 22, *b, c*). The peripheral portion of each cell consists of a lightly staining vacuolated layer, which was formerly, before the pole-cell nuclei separated from the egg, the outermost stratum of the "Keimhautblastem" (Fig. 20, *vac. st.*). This layer has spread completely over the surface of the protruded pole-cells, and in consequence of this increase in area covered, has become thinner; its structure, however, is still the same as when it constituted a part of the egg (Fig. 3, *vac. st.*). This is doubtless due to the granular layer which, by its intervention, impedes the fusion of the vacuolated portion with the denser cytoplasm within the cell.

Just within the outer stratum is the granular layer; this no longer appears evenly distributed (Fig. 21), but now seems to be massed in certain places and absent elsewhere, as shown in Fig. 23, *pdg*. A close examination of sections (Fig. 22, *pdg*) shows, however, that the granules completely surround the nucleus as before but have accumulated in the regions of the pseudopodia. These accumulations are often found in the granular layer of other stages and may even be present in the pole-disc before the pole-cells are protruded (see also Ritter, 1890, Taf. XVI, Fig. 3, and Noack, 1901, Taf. II, Fig. 19).

Between the granular layer and the nucleus is a homogeneous stratum of cytoplasm; this consists of all the protoplasm that surrounded the nucleus before it reached the periphery plus that layer of the "Keimhautblastem" in which the granules were suspended. The nuclei of one of these cells (Fig. 22, *b*) shows a spireme, the other (Fig. 22, *c*) a later stage in which the spireme has already segmented into a number of chromosomes.

As stated above, it sometimes happens that the pole-cell nuclei do not all reach the "Keimhautblastem" simultaneously; those which are delayed have difficulty in collecting enough cytoplasm for their

needs since most of the peripheral layer has already been carried away by the nuclei that first reach the surface. One of these delayed nuclei (Fig. 22, *a*) shows, embedded in its accompanying cytoplasm, a number of small yolk-globules which have not yet been dissolved.

I have already said that eight pairs of pole-cells protrude from the posterior end of the egg and from a single layer there (Fig. 13); these I shall speak of as the primary pole-cells. It has also been shown that when these sixteen primary pole-cells have become completely separated from the egg they are in the prophases of mitosis (Fig. 22). These now undergo their first division, giving rise to the secondary pole-cells which immediately divide again. However, before this second division is completed an accurate count of the number of pole-cells is possible as the presence of granules easily distinguishes them from those of the blastoderm. The number of pole-cells ranges from thirty-two to forty at this stage, thirty-four being present in the specimen illustrated (Fig. 14); only one of these is dividing (Fig. 17) showing that mitosis does not occur in all at the same time. A longitudinal section through an egg taken from the same batch as that just described also shows one pole-cell in a similar condition (Fig. 23, *a*). We conclude from this that the sixteen primary pole-cells have divided, resulting in thirty-two, and that two of these secondary pole-cells have produced daughter cells, thus bringing the total number to thirty-four. During the division of the pole-cells (Fig. 17) all of the granules separate into two approximately equal groups, which form a thin layer closely applied to the cell boundary at either end.

The pole-cells just previous to their second and final division, can, as already mentioned, be distinguished from the adjacent blastoderm-cells by the presence within the cytoplasm of the dark-staining granules of the pole-disc; they also at this stage show a difference in the structure of their nuclei. One cell of each kind, pole and blastoderm, is shown highly magnified in Fig. 28. Here we see that the nucleus of the pole-cell (p.c.) is the larger; it contains a relatively few rod-like pieces of chromatin which are most abundant near the nuclear membrane. In contrast to this, the smaller nucleus

of the blastoderm-cell (bl.c.) is closely packed with chromomeres, and no nucleolus, such as is present in the pole-cell nucleus, is visible.

When the second mitosis is completed, it is difficult to determine the exact number of pole-cells, owing to their irregular arrangement. A superficial view of the posterior end of the egg discloses (Fig. 15) sixty-three pole-cells, one, the sixty-fourth, probably being hidden from sight by overlying cells. In lateral surface view (Fig. 16) the pole-cells are seen as a cap closely applied to the posterior end of the egg. Longitudinal sections (Figs. 23-25) demonstrate that they do not lie free in the polar cavity as described in *Chironomus* (Ritter, 1890, and others), but that those nearest the egg occupy an indentation in its end, in which position they are probably held by the vitelline membrane and the chorion (Balbiani, 1885, in *Chironomus*).

The entire surface of the egg, in the stage just described, is found to be covered by the blastoderm, except the area at the posterior end through which the primary pole-cells passed. Just within this area are seen (Fig. 25) a number of nuclei similar to those of the neighboring blastoderm-cells; they are not, as the latter, separated from one another by cell boundaries, but form an irregular mass, a syncytium. A group of nuclei similar in position and appearance has been noted by Ritter (1890) in *Chironomus* and by Noack (1901) in *Calliphora;* both authors maintain that these are yolk-nuclei. Lecaillon (1898) figures them in an egg of *Lina populi,* but does not mention them in the text (his Fig. 16). Voeltzkow (1889) found a few nuclei in eggs of *Musca* lying in the same region. He calls them inwandering blastoderm-cells. I hope to prove that they are the nuclei of blastoderm-cells, which, owing to the presence of the pole-cells, have been prevented from taking part in the formation of the blastoderm. In the stages figured (Figs. 6-11) no vitellophags are present near the posterior end of the egg from which this group could have arisen. Furthermore, no similar groups of nuclei are to be found at other places in the egg until a much later period of development; then, there may be found irregularly scattered throughout the yolk, small masses of cytoplasm, each containing three or four yolk-nuclei. A comparison of Figs. 23 and 24 shows that in the younger stages

(Fig. 23) the blastoderm ends abruptly at the place where it meets the pole-cells; in the older stage (Fig. 24) it has apparently pushed past the latter on all sides, and now projects obliquely upward into the yolk. A still later stage (Fig. 25) shows that these projecting ends of the blastoderm finally meet, thus forming a structure which in longitudinal section appears as an inverted V, but in reality is a cone-shaped plug extending into the yolk. At first the nuclei are arranged near the surface of this cone in a fairly regular row; some of them, however, soon become displaced. By the time the genesis of the pole-cells is completed, these nuclei form an irregular group, just within the egg opposite the pole-cells with which they are connected by a mass of cytoplasm. We shall see later that they remain thus in communication with the pole-cells for a long period of embryonic growth. *In toto* preparations show this group lying within the yolk in the above described position (Fig. 16, *ps. bl. n.*).

3. *The History of the Pole-Cells Until the Sex of the Embryo Can be Recognized.*

In order to follow the history of the pole-cells, it is necessary to describe the development of the germ-band. The blastoderm, as stated in the last chapter, is present at the conclusion of the pole-cell formation, as a single layer of regularly arranged cells covering the entire surface of the egg, except a small area at the posterior end. The first change noticed in the blastoderm is a crowding together of the cells on the ventral surface of the egg. This results in the formation of a broad longitudinal band of closely aggregated cells, the ventral plate (Stage C). The edges of this plate are thrown up into two folds; these spread out in the posterior region extending to the end of the egg (Stage D), where they pass around the pole-cells and meet on the dorsal surface (Fig. 31). The entire ventral plate now decreases both in length and in breadth; during this contraction a longitudinal concavity, the ventral groove, appears (Stage E). By this shortening of the germ-band, the pole-cells are carried from their former position at the end of the egg (Stage B) to a point slightly anterior, on the ventral surface (Stage E); here they occupy

a cup-shaped depression in the ventral groove. The ventral plate continues to decrease in length; this contraction combined with the above mentioned depression, produces a deep cavity at the posterior end of the groove in which the pole-cells lie (Fig. 29). A lateral view of the same egg (Fig. 30) shows part of the pole-cells concealed within this cavity.

The germ-band can now be recognized; it covers the entire ventral surface of the egg except a wedge-shaped area anterior to the groove (Fig. 29). A lateral view of an *in toto* preparation shows the cephalic region of the germ-band already clearly indicated as a large lateral lobe (Fig. 30). The ventral groove now becomes narrower except at its posterior end; here a comparatively large opening remains (Fig. 32, *a*; Stage F), which, since the last stage (Fig. 29), has moved some distance forward. The floor of the groove has at this point invaginated to produce a cavity which extends obliquely upward into the yolk (Stage G). At the bottom of this cavity we find the pole-cells. They now lie entirely below the surface of the egg, partly hidden under the closely opposed lateral folds (Fig. 32 lf.)

A sagittal section of an egg in this stage gives a good idea of the structure of the ventral plate (Fig. 33). Near the anterior end we can distinguish the beginning of an invagination (*a*) which will become the stomodæum. At the posterior end is noticed a much deeper depression which contains the pole-cells lying at the bottom near the entrance of a distinct canal, the "Polzellencanal" of Escherich (1900). This canal is that opening in the blastoderm at the posterior end of the egg, which was caused at an earlier period (see preceding chapter, Figs. 23 and 25) by the protrusion of the pole-cells. It can be determined from transverse sections that a posterior depression in the ventral groove is formed by the arching over of the lateral folds, thus producing a flask-shaped cavity (Fig. 34, *a*). The pole-cells lie near the pole-cell canal which contains a mass of cytoplasm connecting them with the pseudoblastodermic nuclei within the egg.

The next stage of development (Stage H) shows a still narrower germ-band already displaying signs of segmentation. The posterior

amnioserosal fold has grown forward to cover almost half of the embryo, and is much further advanced than the anterior fold which has just begun to appear at the lateral edges of the cephalic lobes. The flask-shaped depression in the floor of the ventral groove, which was noticed in the preceding stage (Stage G) has increased in depth, forming the posterior amniotic cavity. The posterior end of the germ-band has forced its way through the yolk in this region, becoming a distinct tail-fold (Stage H).

Sufficient *Calligrapha* material was not at hand for a study of sections of this stage (Stage H), but eggs of *Leptinotarsa,* which pass through a similar course of development, were obtained in great abundance; Figs. 35 and 36 were made from eggs of the latter species. A sagittal section of the tail-fold (Fig. 36) shows five pole-cells situated at the end of the amniotic cavity; three other pole-cells have already passed through the pole-cell canal, while another has just commenced its journey. This is the first evidence we have found of the migration of the pole-cells from the outside into the embryo.

A transverse section of another egg (*Leptinotarsa,* Fig. 35) shows a similar migration of the pole-cells; in this preparation a number of pole-cells are lying at the bottom of what was formerly the ventral groove, a few having already entered the pole-cell canal. In both this and the preceding section, the pseudoblastodermic nuclei are fewer in number than in eggs of *Calligrapha.*

An embryo (Stage J) slightly older than that just described exhibits the following changes: first, the amnioserosal folds have almost completely overgrown the germ-band; second, segmentation has become more noticeable; and third, the tail-fold has penetrated still farther forward into the yolk. A sagittal section through the tail-fold (Fig. 37) reveals a well developed mesodermal layer just inside of the ectoderm. A number of pole-cells still remain in the posterior amniotic cavity, although more of them have passed through the pole-cell canal than in the embryo last figured (Stage H). The pseudoblastodermic nuclei which are still present show signs of disintegration.

Such great differences in structure are now apparent between

the pole- and ectoderm-cells that one of each taken from Fig. 37 has been drawn much enlarged (Fig. 55). The pole-cell is nearly spherical; its homogeneous cytoplasm stains lightly; and its nucleus contains besides a nucleolus a number of small groups of chromomeres. The ectoderm-cell, on the other hand, is considerably larger; its cytoplasm stains more deeply and contains several vacuoles, and its nucleus, which is without a visible nucleolus, is completely filled with chromomeres. The relations of the parts of the tail-fold are at this stage most easily understood in transverse sections. In Fig. 38 we can still distinctly see the lateral folds (lf) of the ventral plate; these enclose the flask-shaped ventral groove (a) within which are the pole-cells migrating through the pole-cell canal. The amnion which arches over the structures is separated from the dorsal serosa by a thin layer of yolk. The pseudoblastodermic nuclei are still present within the egg.

The segmentation of the germ-band and the lengthening of the entire embryo now progresses rapidly (Stage K). The cephalic extremity extends completely over the anterior end of the egg, and may be seen covering part of the dorsal surface. The tail-fold has extended a little more than half way up on the dorsal surface of the egg. The segments of the head, thorax and abdomen are visible at this time. The pole-cells have changed their position very little, but a larger number of them are now found inside of the embryo scattered among the mesoderm-cells. A transverse section (Fig. 47) now shows a fairly regular row of pole-cells migrating through the pole-cell canal. The pseudoblastodermic nuclei which have decreased in number by disintegration are represented from this time on only by an occasional nucleus, finally disappearing altogether.

Our next stage (Stage L) is an important one in the history of the pole-cells. The embryo has become more deeply segmented and its appendages are now evident. The tail-fold which has begun to recede is shown in sagittal section in Fig. 40. In most cases all of the pole-cells have by this time migrated within the embryo, but in the section figured two of them are just entering the pole-cell canal which has become much shortened. A transverse section through the last abdominal segment (Fig. 48) reveals the fact that

the pole-cells, as soon as they have entered the embryo, begin to separate into two groups; three of them, in the figure, are present at either side of the median line. A reconstruction made from several series of transverse, sagittal and frontal sections, shows the pole-cells distributed on either side of the last two segments of the tail-fold (Stage L).

The next embryo I shall describe (Stage M) has broadened throughout its entire length; it has also shortened, this being especially noticeable in the posterior portion. This contraction brings the tail-fold nearer the posterior end of the egg than we found it in the previous stage (Stage L). Now that the pole-cells have become an integral part of the embryo I shall call them germ-cells. These still lie in the last two abdominal segments, but have become partially surrounded by mesodermal-cells (Fig. 49). They appear in sagittal section (Fig. 41) to be closer to one another than we found them in Stage L (Fig. 40). In transverse section (Fig. 49) they are shown more clearly separated than before into two groups lying on either side of the tail-fold. Part of them have reached the inner end of the cœlomic cavity, while the others are apparently moving in that direction. Three kinds of cell (germ-, mesoderm- and ectoderm-cells) from Fig. 41 are shown much enlarged in Fig. 57. The germ-cells still exhibit all the characteristics that the pole-cells formerly possessed, and, in addition, contain a second nucleolus. The cells of the mesoderm are smaller; they appear darker than the germ-cells due to a greater susceptibility of their cytoplasm to stains, and the larger relative number of chromomeres in their nuclei. The columnar ectoderm-cells contain cytoplasm which is even darker than that of the mesoderm-cells, and their oval nuclei possess a smaller number of chromomeres, regularly distributed. The ease with which these different cells can be distinguished is evident from a glance at the illustration (Fig. 57).

A transverse section of an embryo slightly older than Stage M (Fig. 50) shows four germ-cells lying in a single row close to one another; they all have now reached a point near the inner margin of the cœlom and have acquired an epithelial covering of mesoderm-cells. A further shortening of the embryo brings the tail-fold close

to the posterior end of the egg (Stage N). The germ-cells, still situated in the last two abdominal segments (Fig. 42), have crowded close together; they do not form, as in younger stages, a loose strand on either side of the body, but now constitute a distinct organ, the germ-gland.

After the contraction of the embryo is completed, the tail-fold no longer exists as such; what was formerly the end of it is now coincident with the end of the egg (Stage O). The posterior abdominal portion of this embryo is shown in sagittal section in Fig. 43. The germ-gland lies between the splanchnic and somatic layers of the mesoderm: its cells have moved closer together forming a more compact organ than before. A frontal section clearly shows its position relative to the other parts of the body (Fig. 54).

Figs. 51, 52 and 53 are from transverse sections of embryos seventy-five hours, eighty-six hours and one hundred five hours old respectively; these illustrate three stages in the path of the germ-glands as they are carried from the ventral to the dorsal side of the body by the lateral growth of the embryo around the yolk. A sagittal section of an eighty-six hour old embryo (Fig. 44) when compared with that of an earlier stage (Fig. 43) also demonstrates the same phenomena.

In the oldest stage figured (one hundred five hours, Fig. 45) it is possible to distinguish the sexes; the male gland is recognized by a constriction which, appearing in its middle region, gives to it a dumb-bell shape; the female organ is distinguished both in transverse (Fig. 53) and in sagittal (Fig. 46) sections by the presence of the developing terminal filaments (*tf.*).

IV. General Considerations.

1. *The Granules of the Pole-Disc.*

So far as I have been able to learn, no author has described in the eggs of Coleoptera a structure in any way corresponding to the pole-disc. Wheeler (1889) failed to note its presence in *Leptinotarsa;* Lecaillon (1898) makes no mention of it in the chrysomelid beetles he studied (see historical part), although in several species

"cellules sexuelles" (pole-cells) were found at the posterior end of the egg. In *Hydrophilus piceus* no record has been made of pole-cells (Heider, 1885, 1889; Deegener, 1900), and, since the primitive germ-cells in this beetle appear at a much later period of development, we should not expect to find a posterior pole-disc in this series. Friederichs (1906) distinguished the primordial germ-cells in *Donacia* shortly after the blastoderm was formed, but found no pole-disc. Saling (1907), who likewise fails to mention these granules, in *Tenebrio,* could not discover as early a segregation of germ-cells as has been found in species of the Chrysomelidæ.

Several other orders of insects have received more attention from embryologists than the Coleoptera, but in only two species, both Diptera, have structures similar to the pole-disc been described. In *Chironomus* Ritter (1890), after giving a brief sketch of the polar bodies and male and female pronuclei, thus continues: "In dem nächsten Stadium sind in dem Dotter keine Zellen mehr zu sehen; dagegen tritt an demjenigen Pol, an welchem später die Polzellen erscheinen, also an dem hinteren, ein eigenthümlicher wulstartiger Körper auf, welcher durch das Hämatoxylin sehr dunkel gefärbt wird. Er erscheint auf mehreren Schnitten und stellt eine etwas nach oben vorgewölbte Platte dar, welche vielfach runde Fortsätze zeigt und aus feinkörnigem Protoplasma besteht. Er bleibt bis zum Austritt der Polzellen an derselben Stelle. Da auf diesem Stadium im Inneren des Dotters keine Zelle mehr zu sehen ist, so kann man nicht umhin anzunehmen, dass dieser Körper den ersten Furchungskern enthält; die dunkle Färbung verhindert aber, dass man denselben mit Sicherheit erkenne.

"Es ist offenbar, dass nach der Theilung des Furchungskernes die Theilprodukte theils in dem dunklen wulstförmigen Körper verbleiben, theils aus demselben herausrücken." Ritter then gives a fragmentary account of the early cleavage divisions of the egg nucleus, at the end of which, the two first pole-cells appear each to contain a "grossen Kern und um denselben herum kranzförmig einen Theil des obengenannten dunklen wulstförmigen Körpers." This author maintains that the "wulstförmige Körper" is intimately concerned with the differentiation of germ-cells from somatic-cells;

he calls this structure the "Keimwulst," a term which had been used by earlier authors to designate very different organs (*e. g.,* Zaddack, 1854), and is too broad in its significance to be appropriate for the pole-disc.

A decade later Noack (1901) figured a similar structure "Polplatte," at the posterior end of the egg of another Dipteron, *Calliphora,* which he designated as the "Dotterplatte." This author holds that at the time the pole-cells appear all the nuclei in the egg are similar. "Es scheint auch thatsächlich die Platte am hinteren Pol die einzige Ursache zur ersten Zelldifferenzirung zu sein." Contrary to what is found in *Calligrapha* this "Dotterplatte" appears in *Calliphora* to impede the progress of the nuclei that encounter it. "Im nächsten Stadium haben die Kerne eine runde Gestalt angenommen, die Platte hat sich in so viel Theile getrennt, als Kerne in ihren Bereich eingetreten sind, und bildet nun um jeden dieser Kerne einen peripher gelegenen feinkörnigen Halbmond. Hiermit ist die erste Zelldifferenzirung eingeleitet." Those cells which now contain granules from the "Dotterplatte" are recognized as pole-cells, while the remaining cells which have reached the periphery of the egg constitute the blastoderm. The "Halbmond" of granules which surrounds the nucleus of each pole-cell now "schliesst sich allmählich zu einem Kreise, welcher um so mehr auffällt, weil die von ihm eingeschlossene und den Kern einbettende Protoplasmamasse fast farblos erscheint (Fig. 25 u. 26, *pz.*). Bei der Fortentwicklung der Polzellen schwindet allmählich die scharfe Grenze zwischen Zellprotoplasma und Polplatte. Letztere löst sich auf und es entsteht eine gleichmässige Pigmentirung, welche den Polzellen noch auf lange Zeit ein ganz charakteristisches Aussehen verleiht." Concerning the nature of this "Dotterplate" Noack says "dass die Platte am hinteren Pole des *Musciden-Eies* sich aus Dotterelementen zusammensetzt. Sie scheint den Zweck zu haben, das Wachsthum am hinteren Pol zu beschleunigen, ferner durch Eintritt in die Polzellen es diesen zu ermöglichen, sich auch weiterhin lebhaft zu vermehren, obgleich sie vom Dotter her keine Nahrung mehr erhalten. Schliesslich verursacht sie die charakteristische Pigmentirung dieser Zellen."

Granular inclusions have also been found in the eggs of other insects, but show only a remote resemblance to the pole-disc granules of *Calligrapha.* Thus Blockmann (1887) discovered a number of bacteria-like rods in the undeveloped eggs of *Blatta germanica.* These rods multiplied by division and were considered true symbiotic bacteria. The same author later described similar "Stäbchen" in several species of Hymenoptera (*Camponotus ligniperdus* and *Formica fusca.* The eggs of *Periplaneta orientalis* and *Ectobia livida* also contain accumulations of "bacterienartige Stäbchen" which later sink into the yolk and disappear (Heymons, 1895).

At the present time, the nature of the pole-disc granules is unknown; one of the two authors quoted above (Ritter, 1890, in *Chironomus*) claims that the pole-disc ("Keimwulst") consists of protoplasm, while the other (Noack, 1901, in *Calliphora*) considers it ("Dotterplatte") to be formed of yolk elements. I hold that the disc is probably derived from the nucleus during the growth of the oögonia.

The origin of these granules may be difficult to determine, although it seems not an impossible undertaking. Lack of material has prevented me from tracing them in stages earlier than eggs of *Leptinotarsa* just previous to laying, at which time the polar bodies are being formed (Fig. 2); here, however, the pole-disc is as large as in later stages of development and it could doubtless be found in younger eggs.

A number of facts discovered in other invertebrates may be mentioned in favor of the nuclear origin of the granules of the pole-disc. Blochmann (1886), Stuhlman (1886) and others have described for various species of Hymenoptera a budding of the nucleus in the oöcytes; these buds result in the formation of many small "nuclei" ("Nebenkerne," Blochmann; "Dotterconcretionen," Stuhlmann) each containing small dark-staining granules. The "nuclei" thus derived from the nucleus of the oöcytes pass out to the periphery of the cell and are lost to view. No pole-disc has been recorded in any of these species of Hymenoptera, but in the Dipteron, *Musca vomitoria,* where a pole-disc probably does occur, Korschelt (1886) has described bodies in the oöcytes similar to the "Nebenkerne" of

Blochmann. It is highly probable that a similar expulsion of nuclear material and corresponding decrease in the size of the germinal vesicle takes place in the oöcytes of Chrysomelid beetles, and that the particles of chromatin thus set free gather at the posterior end of the egg to form the pole-disc.[1]

Wheeler's (1897) theory to account for the presence of dark-staining granular inclusions within the eggs of *Myzostoma glabrum* suggests that the granules of the pole-disc may be derived from the nuclei of the nurse-cells which, in many insects, pass into the early oöcytes.

The results that Häcker (1897) obtained from a study of the "Keimbahn" in *Cyclops* also point to a nuclear origin of the pole-disc granules. According to this author "Aussenkörnchen" arise at one pole of the first cleavage spindle; these are derived from disintegrated nucleolar material and are attracted thus to one pole of the spindle by a dissimilar influence of the centrosomes. During the first four cleavage divisions the granules are segregated always in one cell; at the end of the fourth division these "Aussenkörnchen" disappear, but the cell which contained them can be traced by its delayed mitotic phase into the primitive germ-cells. The "Aussenkörnchen" found in the germ-cell antecedents of *Cyclops* show a remarkable resemblance to the pole-disc granules of *Calligrapha;* one important difference, however, may be pointed out, *i. e.,* the fact that in the former the granules arise from the nucleolar material

[1]Since this paper was sent to press an account of the origin of the primordial germ-cells in some parasitic Hymenoptera has appeared which furnishes proof of the nuclear origin of a structure similar to the pole-disc. I refer to the work of F. Silvestri, entitled *Contribuzioni alla conoscenza biologica degli imenotteri parassiti,* published in the *Bollettino del Laboratorio di zoologia generale e agraria* della R. Scuola Superiore d' Agricoltura di Portici, Vol. 3, April, 1908. The following quotation gives some of the results of his study of *Ageniaspis fuscicollis.* "Che il nucleolo durante la formazione dei globuli polari si conserva immutato nella parte posteriore dell' ovo e che passa intero ad una delle prime due cellule di segmentazione. Tale nucleolo, come nel *Litomastix truncatellus,* ha un' azione ritardatrice della divisione della cellula, in cui si trova ed è da ritenersi, per quanto ho anche osservato nello sviluppo delle due specie, di cui appresso tratto, un determinante della celulle germinali" (p. 53).

of both male and female pronuclei, after, or during their conjugation, while in Chrysomelid beetles the pole-disc is already fully formed before fertilization.

Another interesting variation in the origin of the germ-cells was described by Boveri (1892) in *Ascaris*. After the first cleavage division of the egg of this Nematode one cell preserves its chromosomes intact, while the other casts off the swollen ends of its chromosomes into the cytoplasm. During the first five cleavage divisions, one cell retains the two full chromosomes, while all the others contain the reduced amount. At the thirty-two cell stage, the cell containing the two full chromosomes is given up entirely to the production of the reproductive organs; the other cell gives rise only to somatic tissue.

The value of the pole-disc in the development of the insect egg can only be surmised. Ritter (1890) maintains that the first cleavage nucleus is hidden among the granules of the "Keimwulst," and he is inclined to believe that the first cleavage division separates the primitive germ-cell substance from the somatic material, although he could not demonstrate this. Noack (1901) suggests that the yolk elements of the "Dotterplatte" may hasten the growth at the posterior pole of the egg, and that later they may possibly increase the vigor of the pole-cells. That the pole-cells need special means of nourishment is doubtless the case, for contrary to the condition in the blastoderm-cells they are, at an early period, entirely separated from the yolk, and later use up energy in their migration.

Primary pole-cells are evidently characterized by the presence of yolk material, as may be illustrated by the following citations. In *Chironomus nigro-viridis* Weismann (1863) found four oval nuclei lying in the "Keimhautblastem" at the posterior end of the egg; each of these, he says, "besassen einen kreisrunden, klaren, etwas röthlich schimmernden Kern, und in einigen Lagen ausserdem noch ein oder zwei Dotterkörnchen." These are the "Polzellen." In another Dipteron, *Simula sp.*, Metschnikoff (1866) records four or five pole-cells which "bestehen ausser einem Kerne noch aus einer die feinsten Dotterkörnchen enthaltenden Zellsubstanz." The same author (1866) also states that when the pseudovum in the

pædogenetic larva of *Miastor* contains twelve to fifteen nuclei, "Man bemerkt zunächst, dass der am spitzen Pole des Pseudovums liegende Keimkern von einer dicken dunkeln Dottermasse schärfer umgeben wird und mit dieser zusammen bald in eine besondere, 0.017 mm. grosse, membranlose Zelle sich abschnürt." This gives rise to the pole-cells. Schwangart (1905) found that the primitive germ-cells of *Endromis* "sind grösser und dotterreicher" than the blastoderm-cells.

The theory that the yolk contained in the germ-cells is transformed by them into the energy of motion is strengthened by the fact that the migrating germ-cells of many species of vertebrates also contain yolk-globules (Beard, 1902, in Elasmobranchs; Eigenman, 1892, in Teleosts; Nussbaum, 1880, in Amphibia; Allen, 1906, in Reptilia).

2. *The Migration of the Primitive Germ-Cells in the Insecta.*

A. *The Migration of the Pole-Cells through the Pole-Cell Canal.*

The authors who first described the pole-cells of insects (Robin, 1862; Weismann, 1863) supposed that they took part in the formation of the blastoderm. This interpretation was corrected by Metschnikoff (1865) and Leuckart (1865) who maintained that the pole-cells develop into the germ-gland. These two authors, however, did not tell us how the primitive germ-cells got back into the egg after their complete separation at the posterior end. Balbiani (1882-5) added the evidence necessary to prove that the pole-cells really become the reproductive organs, but he was unable to determine whether they force their way through the blastoderm, or pass into the egg by way of a clear space left for their entrance. Sections of the eggs of *Chironomus* led Ritter (1890) to believe that the pole-cells move of their own accord through a gap in the blastoderm which then closes after them.

Escherich (1900) in *Musca* made the first accurate study of the passage of the pole-cells from the posterior amniotic cavity into the embryo. Many of his figures show conclusively not only "die Wanderung der Polzellen durch die Keimplatte" but also an "intercellular

Canal der den Polzellen den Durchtritt ermöglichte." In his Fig. 55 several pole-cells are seen lying in the groove outside of the germ-band, several are present within it and a number are represented half way through the opening in the "Entoderm," the "Polzellencanal." This migration of the pole-cells extends over a considerable period. Escherich claimed that since the region of the ventral plate containing the "Polzellencanal" later constituted a part of the "ventrale Wand des Urdarmes, . . . somit ist es uns dadurch möglich, die Mittelplatte als erste Anlage des Entoderm zu erkennen." In the last embryonic stage examined by this author, all of the pole-cells had not yet completed their migration.

Noack (1901), in *Calliphora,* also records the migration of the pole-cells from the dorsal groove of the germ-band into the embryo. This author, contrary to what Escherich found, could not satisfy himself that a definite canal exists, but decided that the pole-cells during their migration produce an adventitious gap in the "Entoderm." Noack followed the history of the pole-cells until they became scattered among the entoderm cells within the embryo.

Lecaillon (1898) is the only author who has described pole-cells in the Coleoptera. He states that after the "cellules sexuelles" are separated from the posterior end of the egg they "ne restent d'ailleurs pas longtemps en dehors de la masse ovulaire; à mesure que la segmentation progresse et que l'enveloppe blastodermique se complète, elles commencent à rentrer dans l'œuf. Pour cela, elles refoulent le vitellus devant elles et s'insinuent entre les cellules périphériques du pôle postérieur de l'œuf, au fur et à mesure que celles-ci émergent de la masse vitelline." When the blastoderm is finally completed, the "cellules sexuelles" for a group "entre le vitellus et l'enveloppe blastodermique," as indicated in his Fig. 10. In only one of the species studied, *Gastrophysa raphani,* did a variation from this order of events occur; in this form the "cellules sexuelles," although tending to re-enter the egg, remain outside, as we have found the pole-cells to do in *Calligrapha* and *Leptinotarsa.* Their migration was not carefully followed by Lecaillon in *Gastrophysa.* He found them lying at the end of the posterior amniotic cavity, and shows two of them in his Fig. 22, "dans le sillon profond qui se trouve sur le

milieu de sa paroi interne;" these "pénètreront au milieu des cellules mésodermique au moment où le sillon se fermera." No pole-cell canal is figured, nor does he mention in the text by what means this penetration takes place.

Although Friederichs (1906) did not observe the formation of pole-cells in *Donacia crassipes* he found a group of germ-cells lying just within the egg at the posterior pole; these, he thinks, are derived from the blastoderm. Beneath this group is an opening in the blastoderm similar to the pole-cell canal found in *Calligrapha,* and one cannot but suspect that pole-cells arise in *Donacia* as in other Chrysomelidæ, but were overlooked by this author. The opening in the blastoderm is considered the blastopore. Friederichs says of it, "Der Blastoporus der Chrysomeliden liegt am hinteren Pol und wird verschlossen durch die Genitalanlage, welche an dieser Stelle bereits ensteht, sobald das Entoderm (die primären Dotterzellen) und das primäre Ektoderm gesondert sind. Der Blastoporus wird später zum After des Insekts."

In *Calligrapha* and *Leptinotarsa,* a definite pole-cell canal is present, homologous to the "Polzellencanal" found by Escherich in *Musca.* The origin of this canal has been described in a previous chapter (III) and the progress of the pole-cells through it was there followed in detail. It undoubtedly is the opening in the blastoderm caused by the pole-cells during their formation; this opening is kept free from cells by a plug of cytoplasm connecting the group of pole-cells lying in the posterior amniotic cavity with the pseudoblastodermic nuclei just within the egg (Fig. 25). The canal closes only after all the pole-cells have passed through it (Fig. 40). It will be shown later that in *Calligrapha* the pole-cells migrate into the embryo by means of amœboid movements.

B. *The Migration of the Germ-Cells within the Embryo.*

It is difficult to ascertain in many cases whether the germ-cells described by the earlier authors changed their position within the embryo by means of an active migration, or were passively moved about by the shifting of the other embryonic tissues. Metschnikoff

(1866), Balbiani (1885) and Ritter (1890), in the Diptera, and Huxley (1858), Metschnikoff (1866), Balbiani (1866), Witlaczil (1884) and Will (1888), in the Hemiptera, describe a precocious segregation of the germ-cells. In the Diptera no active migration is reported after the pole-cells re-enter the egg; in the Hemiptera no movements have been noted at any stage in the history of the germ-cells.

The primitive germ-cells of several species of Orthoptera are, when first seen, in the act of penetrating the walls of the cœlomic cavities (*Blatta,* Cholodkovsky, 1891, and Heymons, 1891; *Xiphidium* Wheeler, 1893). As noted elsewhere in this paper, Heymons (1895) has established a much more extensive migration of the germ-cells in *Blatta* and other allied forms than was described by the authors named. A migration similar to that found in *Blatta* occurs in *Forficula* (Heymons, 1895).

The migration of the germ-cells in the Aptera has been recorded by two observers, Heymons (1897) and Claypole (1898). The former found that the germ-cells in *Lepisma* have an ectodermal origin at the posterior end of the germ-band. They soon "sich zerstreuen und einzeln, zwischen und neben den Mesodermzellen nach vorn wandern." The germ-cells are less favorable for study in *Lepisma* than in *Periplaneta,* but Heymons nevertheless convinced himself "dass die Wanderung der Geschlechtszellen sich ganz ähnlich wie bei den Orthopteren vollzieht." According to Claypole (1898) the germ-cells of *Anurida* move into the yolk at a late embryonic stage and begin to mingle with its globules. Under high magnification "the peculiarly 'succulent' character of the cells" could be seen.

Carriere and Burger (1897) describe in the Hymenopteron, *Chalicodoma,* the migration of the germ-cells from the third and fourth abdominal segments into the fifth.

In the "drone eggs" of the bee, the germ-cells penetrate the walls of the primitive somites and congregate in the cœlomic cavities. (Petrunkewitsch, 1903).

Although Woodward (1889) correctly observed the place of origin of the germ-cells in the Lepidopteron, *Vanessa,* it remained for Schwangart (1905) to follow their further history. In *Endromis*

the germ-cells separate into groups which migrate from a point near the posterior end of the egg, to the fourth, fifth, and sixth abdominal segments.

As soon as the pole-cells of *Calligrapha* have passed through the pole-cell canal, they loose their pronounced pseudopodia-like processes and become nearly spherical (Fig. 55); nevertheless, they undergo a decided change in position. They move away from the inner end of the pole-cell canal, and creep along between the yolk and the germ-band (Figs. 47-49). Thus two groups are formed near the developing cœlomic sacs; each group probably contains an equal number of cells. The smallest number I have counted in one group at this time (Stage M) is thirty; the largest number, thirty-four. As there is some difficulty in obtaining an accurate count, it seems probable that the sixty-four germ-cells are equally divided and that each germ-gland receives thirty-two. Some of the germ-cells migrate not only laterally along the germ-band but also back toward the posterior end of the egg, where we find them forming narrow strands in the last abdominal segments (Figs. 39-41). From this stage on, the germ-cells are not very active; they move closer to one another to form the compact germ-glands. I was unable to determine whether the later movements of the germ-cells are due to an active migration, or to the tensions created by the growth of the surrounding tissues; the latter seems the more probable.

C. *The Method of Locomotion of the Germ-Cells.*

Nearly all of the authors, who have observed the migration of the germ-cells in the Insecta, have failed to describe their method of locomotion. Thus Robin (1862) and many of his contemporaries state that in the Diptera, the pole-cells, shortly after their appearance, move back into the egg. These authors, however, give no explanation to account for these movements, and one is not enlightened as to whether the pole-cells are passively carried from place to place, or whether they undergo an active migration. That amœboid movements might possibly explain the re-entering of the egg by the pole-cells, was first suggested by Weismann (1882). He was unable to follow the history of these cells in *Chironomus,* because they dis-

appeared from view beneath the egg. One cell lying near the anterior end of the egg was capable of amœboid movements, but its identity was not established. Ritter (1890), after finding that the pole-cells in *Chironomus* penetrate the blastoderm, concludes that "anders als durch aktive Bewegung können sie wohl kaum in die Lage kommen, in welcher wir sie Fig. 10 finden." The cells in the figure mentioned have, however, a spherical form exhibiting no pseudopodia-like processes.

Turning now to the Coleoptera, we find sufficient evidence to prove that the pole-cells migrate by means of amœboid movements. Although Wheeler (1889) failed to find the pole-cells in the very early stages of *Leptinotarsa,* he figures several of them (his Fig. 82) in a sagittal section of an egg carrying a segmented germ-band. Here are shown three cells "which are on the surface of the embryo in the amniotic cavity. They are very large and clear and the more anterior is apparently creeping in the manner of an Amœba, along the surface of the abdominal ectoderm. These cells, the ultimate fate of which I have been unable to determine, probably escape from the anal orifice of the gastrula before it closes." This author also shows in transverse section (his Fig. 87) a cell which, he says, is "about to wander through the blastopore into the amniotic cavity." He suggests that this may be the homologue of the "Polzellen." My sections prove that these are really pole-cells and that they creep along the surface of the ectoderm by amœboid movements, but the direction of their migration is the reverse of that stated by Wheeler, *i. e.,* they are not wandering outward into the amniotic cavity but are on their way into the embryo.

In *Clytra* and other species of Chrysomelid beetles, Lecaillon (1898) finds that the "cellules sexuelles," as he designates the pole-cells, migrate back into the egg shortly after their appearance. In another species (*Gastrophysa raphani*) studied by this author, these cells remain outside of the egg until a later stage of development, and then they penetrate the "ectoderm." Lecaillon does not present any evidence to account for this migration, he says, however, that these cells "se montrent en général moins bien fixées que les autres cellules." In my material no difficulty was experienced in obtaining

perfectly fixed pole-cells, and I conclude that Lecaillon was deceived by the irregular outline of the "cellules sexuelles," and that in Clytra the apparent distortion of these cells was due, not to poor fixation, but to their amœboid character.

Several authors have described the locomotion of primitive germ-cells in other orders of insects. Ayers (1883) states that the germ-glands of *Oecanthus* "are first seen as two irregular groups of amœboid cells." In *Forficula* (Heymons, 1895) the germ-cells arise near the posterior end of the egg and migrate anteriorly. "Die Bewegung dürfte hierbei durch Aussenden amöboider Fortsätze erfolgen, die man jetzt an den Zellen gar nicht selten beobachten kann."

Heymons (1895) says of *Periplaneta,* "Aehnlich wie in gewissen Stadien von *Forficula* scheint die Fortbewegungsart der Zellen hierbei eine amöboide zu sein, es kann dies wenigstens aus den zahlreichen Gestaltsveränderungen der Geschlechtszellen geschlossen werden, die bald rundlich, bald langgestreckt sind oder lappige Fortsätze aussenden."

The pole-cells of both *Calligrapha* and *Leptinotarsa* not only migrate by their own activity, but their movements are distinctly amœboid. It has been noted above that every preblastodermic nucleus has long cytoplasmic processes extending out on all sides into the yolk. It has also been shown that these processes become blunt in the case of the pole-cells when separation from the egg takes place (Fig. 22). If we examine the pole-cells from the time they are protruded until they become aggregated into a distinct germ-gland, we discover a series of stages which establish their amœboid character as well as it is possible to do in fixed material.

During their protrusion, the pole-cells have still an irregular outline, but their cytoplasmic processes are no longer present on their outer surface; this is probably due to being pressed against the "Keimhautblastem" (Figs. 20 and 21). After complete separation, however, they again acquire an amœboid shape, their blunt pseudopodia containing most of the granules taken from the pole-disc (Fig. 22). This appearance is retained until the pole-cells begin to migrate through the pole-cell canal; then the pseudopodia

are no longer found on all sides of the cell, but are definitely directed toward the entrance to the canal. This may be seen in a transverse section of an egg of *Leptinotarsa* similar to Stage H (Fig. 35). In this figure two pole-cells are near the inner end of the pole-cell canal, two are creeping along one side of the groove in the germ-band, while three others are still in the amniotic cavity, evidently moving toward the canal. A greater magnification brings out more clearly the shape of the pole-cells and the direction of their migration. Fig. 56 shows two of these cells taken from a transverse section (Fig. 38) of the tail-fold of an embryo similar to Stage J. The pseudopodia are here unmistakable; they are extended toward the entrance to the pole-cell canal. The hyaline cytoplasm at the tips of the pseudopodia resembles the ectoplasm of Amœba; it will be recognized as the vacuolated layer which was carried away from the "Keimhautblastem," where the pole-cells were extruded from the egg (Fig. 21, vac. st.). The pole-cells seem to be thigmotactic, few of them being found free in the amniotic cavity; they are usually observed close to the germ-band or crowded one against another.

Fig. 55 shows a pole-cell and an adjacent blastoderm-cell enlarged from the sagittal section shown in Fig. 37. Here we find little or no evidence of pseudopodia. This is the usual condition of the pole-cells after they have reached the interior of the egg. Their method of progression from this stage on is not easily made out. In a later stage (Stage M) the germ-cells are partly surrounded by mesoderm-cells; their outline is still irregular, as is shown in the enlarged drawing (Fig. 57), but no long pseudopodia such as are present in the younger embryos can be seen. It may be that the pole-cells cease to move actively after they reach the interior of the embryo, and that they are pushed into place by the rapidly proliferating mesoderm-cells.

3. *The Origin and Early History of the Germ-Cells in the Insecta.*

No general statement can be made regarding the time and place of origin of the primitive germ-cells in the Insecta, as the species which have been examined represent only a small proportion of the types necessary for a thorough understanding of this subject.

Petrunkewitsch (1901 and 1903) has described the origin of the primitive germ-cells in the "drone eggs" of the bee at a period earlier than has been recorded for any other insect. The inner half of the first polar body of these unfertilized eggs unites with the second polar body to form the "Richtungscopulationskern" which is the primordial germ-cell. Weismann (1904) vouches for the exactness of Petrunkewitsch's results. In other Hymenoptera no germ-cells have been found previous to the appearance of the mesoderm (Carriere and Burger, 1897).

The primitive germ-cells (pole-cells) of several species of Diptera have a very early origin. Weismann (1904), discussing the development of the reproductive cells in connection with his "germ-plasm theory," says: "If we could assume that the ovum, just beginning to develop, divides at its first cleavage into two cells, one of which gives rise to the whole body (soma) and the other only to the germ-cells lying in this body, the matter would be theoretically simple. . . . As yet, however, only one group of animals is known to behave demonstrably in this manner, the Diptera among insects. . ." I have been unable to find in embryological literature any account of such a phenomenon in this order of insects. The pole-cells of Diptera are always found at the posterior end of the egg. The time of their first appearance varies in the different species described. In *Miastor* the primordial pole-cell nucleus can be distinguished when there are only eight to fifteen nuclei in the pseudovum (Leuckart, 1865; Metschnikoff, 1865). In *Chironomus* the single primordial pole-cell appears before the blastoderm is formed and is closely followed by a second, these two then divide, re-enter the egg, and develop into the germ-glands (Grimm, 1870; Weismann, 1882; Jaworowski, 1882; Balbiani, 1885; Ritter, 1890). The first pole-cell nucleus in this species may divide before it separates from the egg. Weismann (1863) noted four pole-cell nuclei lying in the "Keimhautblastem," while several authors have described the appearance of two, before separation takes place (Grimm, 1870; Weismann, 1882).

In other species of Diptera the primordial pole-cell nucleus apparently divides several times before it reaches the surface of the

egg; there are fifteen to twenty in *Calliphora* (Noack, 1901), and four to five in *Simula* (Metschnikoff, 1866) and in *Pulex* (Packard, 1872), although neither of the latter was examined carefully.

Pole-cells are also found in several Chrysomelid beetles. Lecaillon (1898) made no attempt to count the number of "cellules sexuelles" in *Clytra,* but states that they are the cells which first reach the "Keimhautblastem" at the posterior end of the egg. In *Calligrapha* I have shown that the primitive pole-cell nuclei may be recognized when they are four in number, but that these divide twice before they separate from the egg, *i. e.,* there are sixteen which pass through the pole-disc. After separation these divide twice, giving rise to sixty-four. This number remains constant until the embryo is nearly ready to hatch (Fig. 45); then the germ-cells increase rapidly by mitosis.

The very early stages of pole-cell formation were not observed by me in *Leptinotarsa.* When the pole-cells were first seen in this species, they formed a group lying at the posterior end of the egg (Fig. 26); they are in every way similar in appearance to those found in *Calligrapha* (Fig. 24, Stage B), being amœboid in shape and containing a layer of granules which they have gathered from the pole-disc (Fig. 2). Embryos similar to Stages A to O were examined and in every case the germ-cells were discovered occupying a position which corresponds almost exactly to that found in *Calligrapha.* Wheeler (1889), in his work on *Leptinotarsa,* not only failed to find the pole-disc, but also overlooked the pole-cells at the posterior end of the egg. His Figs. 66, 67 and 70 represent surface views of embryos like my Stages C, D and F, of *Calligrapha.* The group of pole-cells is present in every one of these stages in *Lepinotarsa,* and I cannot understand why Wheeler failed to find them. On page 321 Wheeler (1889) says: "Sections taken in all directions through the egg show the blastoderm to be of even thickness over the whole surface (Fig. 63)." This is not true of the eggs of *Leptinotarsa* I have examined, as there are two or three layers of cells at the posterior end at the stage to which he refers. The space beneath the pole-cells remains free from blastoderm-cells, and later becomes the pole-cell canal just as we found in *Calligrapha.*

The primitive germ-cells may be recognized in the parthenogenetic eggs of Aphids shortly after the blastoderm is completely formed. Some authors were able to trace them back to a single cell which separates from the inner surface of the blastoderm near the posterior end of the egg (Balbiani, 1866; Witlaczil, 1884; Will, 1888).

The only investigators who have recorded an early appearance of the primitive germ-cells in the Leptidoptera are Balbiani (1869-72), Woodworth (1889) and Schwangart (1905). These authors found a thickening of the blastoderm near the posterior end of the egg. The inner cells of this thickening differentiate into germ-cells; later these migrate singly into the fourth to the eighth abdominal segments (Schwangart, 1905).

The foregoing accounts show that those embryologists who hold that the germ-cells in the Insecta have a mesodermal origin are not in harmony with the results obtained by recent investigators. Heymons (1891), Korschelt and Heider (1892), and Wheeler (1893) all regarded the primitive germ-cells of Diptera and Hemiptera "as derived by a process of precocious segregation from metameric gonads like those of the Orthoptera" (Wheeler, 1893). The germ-cells in the Orthoptera (*Blatta* and *Periplaneta*), however, do not arise metamerically from the mesoderm and later migrate into the primitive somites (Heymons, 1895).

As noted above, the stage of embryonic development in which the germ-cells can first be recognized varies considerably in different species of insects. By the majority of authors the reproductive cells have been considered of mesodermal origin, by others they are supposed to arise from ectoderm-cells, blastoderm-cells, yolk-nuclei, or early cleavage nuclei. I believe with Woodworth (1889) that "the germinal cells do not belong to any layer, but are the products of the first divisions of the egg cell; they take part generally in the formation of the blastoderm and then migrate into the mesoderm. . . . In all cases where they are supposed to come from the mesoderm, the later stages comparatively are the only ones known." Heymons (1893) four years later was led to similar conclusions. This author states "dass die Geschlechtszellen der Insekten überhaupt nicht von diesem oder jenem 'Keimblatte' abzuleiten sind, sondern

nur scheinbar je nach dem Zeitpunkt ihres Hervortretens bald dieser, bald jener Zellenschicht angehören.

"Wenn auch die Trennung zwischen somatischen Zellen und Geschlechtszellen bei den meisten Insekten erst spät bemerkbar wird, so werden wir somit doch annehmen müssen, dass ein solcher Unterschied bereits vom Beginne der Entwicklung an vorhanden ist.

"Es mag noch hervorgehoben werden, dass die Geschlechtszellen der Insekten nicht, wie man bisher geglaubt hat, in metamerer Anordnung in den einzelnen auf einander folgenden Abdominalsegmenten zur Anlage kommen, sondern dass ihr Ursprung am hintersten Ende des Keimstreifs zu suchen ist, von wo aus erst im Laufe der Entwicklung eine Wanderung oder Verschiebung nach vorn hin erfolgt. Dies trifft zunächst für die hier beschriebenen Formen zu, hat möglicherweise aber für sämmtliche Insekten Gültigkeit." Heymons is an investigator who has not been content to work on a few widely separated types of insects, but has made comparative studies of nearly allied species. The value of this kind of research is strikingly shown by the results he obtained from a study of *Blatta* and *Periplaneta*.

In an early paper Heymons (1891) stated that the germ-cells of *Blatta* are derived from the mesoderm just previous to the segmentation of the germ-band. Later (1895) *Periplaneta* was also examined. In this Orthopteron the germ-cells were found to arise from the ectoderm at the posterior end of the egg; they separate from one another, migrate anteriorly, and arrange themselves intersegmentally. A re-examination of *Blatta* convinced Heymons that the germ-cells originate in this cockroach as they do in *Periplaneta,* but can be distinguished from the mesoderm-cells only when they reach the primitive somites. This is made more certain by the discovery of a similar origin and migration in *Forficula* (Heymons, 1895) and *Lepisma* (Heymons, 1897).

In *Calligrapha* all the nuclei of the egg are apparently alike, potentially, until in their migration toward the surface they reach the "Keimhautblastem;" then those which chance to encounter the granules of the pole-disc are differentiated by their environment, *i. e.,* the granules, into germ-cells. In other words, whether or

not a cell will become a germ-cell depends on its position in the egg just previous to the formation of the blastoderm. The cleavage nuclei of the beetle's eggs are not separated by cell boundaries, as are those of several other animals (*e. g. Cyclops* and *Ascaris*), where an earlier differentiation of the primordial germ-cells takes place, but are intimately connected by the cytoplasm which is present throughout the egg in the interdeutoplasmic spaces. We have thus a syncytium in which the nuclei are widely separated from one another by the enormous mass of yolk. The various substances (*e. g.*, the granules of the pole-disc) are, therefore, less easily segregated into a single cell in the egg of *Calligrapha* than are similar structures (*e. g.*, the "Aussenkörnchen" of *Cyclops*, Häcker, 1897) in alecithal eggs. This fact may account for the relatively late stage at which the primitive germ-cells of *Calligrapha* and allied forms can be recognized as such.

V. Summary.

1. A layer of dark-staining granules, the pole-disc, is present at the posterior end of the eggs of *Calligrapha* and *Leptinotarsa* before fertilization takes place; this layer is later intimately associated with the development of the pole-cells.

2. The genesis of the pole-cells is as follows: (1) four nuclei lying near the posterior end of the egg are recognized by their position as pole-cell antecedents (Figs. 5 and 12); (2) these four nuclei divide producing eight daughter nuclei which move closer to the periphery of the egg (Fig. 7); (3) these in turn divide resulting in sixteen nuclei, arranged in pairs, each of which separates entirely from the egg, carrying with it a portion of the "Keimhautblastem" containing pole-disc granules (Figs. 13 and 19-22); (4) the sixteen primary pole-cells divide to form thirty-two secondary pole-cells (Fig. 14); these divide resulting in sixty-four tertiary pole-cells (Figs. 15-16) which do not increase in number until a late period of embryonic life (Fig. 45); (5) in mitosis the pole-disc granules are, approximately, equally distributed between the two daughter cells (Figs. 17 and 27 *a*).

3. That area of the egg through which the pole-cells pass is not closed by the blastoderm but becomes the pole-cell canal, through which the pole-cells later migrate into the embryo (Figs. 33-40).

4. The blastoderm-cells which fail to cover this area form a syncytium containing pseudoblastodermic nuclei; these nuclei for a long period lie just within the egg near the pole-cell canal, and finally disintegrate (Figs. 24-25 and 33-39).

5. After their separation from the egg the history of the pole-cells is as follows:

(1) The pole-cells are carried slightly forward on the ventral surface of the egg by the contraction of the ventral plate (Fig. 29, Stage E); (2) they sink into the posterior depression of the ventral groove, which is the beginning of the posterior amniotic cavity (Figs. 33-34, Stages F-G); (3) they are carried along by the developing tail-fold, which penetrates dorso-anteriorly into the yolk (Stages H-K); (4) they migrate through the pole-cell canal into the embryo by means of amœboid movements (Figs. 35-40, 47, 56); (5) upon reaching the interior of the embryo they separate into two groups, which come to lie as a strand on either side of the body, in the last two abdominal segments (Figs. 40, 47-49 and Stages K-L); (6) these two strands become shorter by a crowding together of the germ-cells (Fig. 41, Stage M); (7) each of the two germ-glands thus produced acquires an epithelial covering of mesoderm-cells (Figs. 42, 50); (8) the germ-glands, situated as before in the last two abdominal segments, are carried, by the shortening of the embryo, to a ventral position on either side of the body (Figs. 42-43, Stages M-O); (9) by its lateral growth around the yolk, the embryo carries the germ-glands to a point near the dorsal surface on either side of the mid-intestine (Figs. 51-53, 43-45); (10) the sexes can be distinguished at this time by the shape of the germ-glands, that of the male being dumb-bell shaped (Fig. 45), while the female reproductive organ is pear-shaped, and shows the development of terminal filaments (Figs 46 and 53).

VI. Material and Methods.[2]

The eggs of Chrysomelid beetles are usually laid on the leaves of the plants, which serve as food for the larvæ. *Callagrapha multipunctata* deposits its eggs in batches of from two to twenty on the under surface of willow leaves (*Salix longifolia*). A number of these beetles were kept in stender dishes and their eggs as soon as laid were transferred to watch glasses. All the eggs laid at one time were found to be in practically the same stage of development, and the batches were, therefore, carefully separated from one another. The age of the eggs proved to be no exact criterion of their developmental progress as external factors (temperature, humidity, etc.) play an important part in the rapidity of embryonic growth. Thus, two eggs of the same age which are kept under different environmental conditions, may on examination be found in very different stages of development. For this reason the various embryos figured are not designated by the number of hours since the eggs were laid, but are classed arbitrarily according to their stage of development.

Eggs of *Calligrapha lunata* were found on leaves of the wild rose (*Rosa blanda*); those of Leptinotarsa were taken in abundance from potato plants (*Solanum tuberosum*). The eggs were preserved at intervals of from fifteen minutes to one hour and a complete series was obtained from those just laid to those containing embryos ready to hatch. A few eggs of *Leptinotarsa* were dissected out from the oviducts of the adult beetles.

A large number of fixing fluids were tried; the one which gave the best results was a modification of Petrunkewitsch's fluid. The mixture was heated to a temperature of about 80° C., and poured over the eggs; this fluid was followed after half an hour by seventy per cent alcohol containing a small amount of iodine. After the above fixation, the chorion stood away from the egg so that it could easily be removed with needles under the binocular microscope. Sections were cut $6^2/_3$ microns thick and were stained on the slide; Mayer's acid-hæmalum followed by Bordeaux red, was used more than any other combination, although most of the commoner methods

[2]For an account of the breeding habits of these beetles see Hegner, 1908.

were employed in checking the results. The germ-cells stained deeply in orange G, and in picric acid, and could be distinguished without difficulty by their affinities for these colors. Entire embryos were stained in Conklin's picro-hæmatoxylin, Mayer's acid-hæmalum or Partsch's alum cochineal; some remarkably clear preparations of the posterior end of the egg were procured by overstaining in acid-hæmalum, decolorizing in absolute alcohol containing one per cent HCl, and then immediately clearing in xylol, and mounting. By this method the density of the hæmatoxylin was reduced and a transparent reddish hue remained; the granules of the pole-disc and nuclear structures could be clearly distinguished in thick preparations after this treatment.

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EXPLANATION OF PLATES.

Reference Letters.

am., amnion.
am.s., amnioserosal fold.
ap., appendage.
bl., blastoderm.
bl.c., blastoderm-cell.
bl.c.n., blastoderm-cell nucleus.
ch., chorion.
coe., coelomic cavity.
d.y., disintegrating yolk-globule.
ec., ectoderm.
e.m.i., epithelium of mid-intestine.
f.b., fat-body.
g.bd., germ-band.
g.c., germ-cell.
g.gl., germ-gland.
K.h.bl., "Keimhautblastem."
l.f., lateral fold of ventral groove.
mp.t., Malpighian tubule.
ms., mesoderm.
m.t., muscular tissue.
n.s., nervous system.
p.am.c., posterior amniotic cavity.
p.bl.n., preblastoderm nucleus.
p.c., pole-cell.
p.c.c., pole-cell canal.
p.c.n., pole-cell nucleus.
p.d., pole-disc
p.d.g., pole disc granule.
ps.bl.n., pseudo-blastodermic nucleus.
s.m., splanchnic mesoderm.
so.m., somatic mesoderm.
sr., serosa.
t.f., terminal filament.
tr., tracheal invagination.
y., yolk.
vac., vacuole.
vac.st., vacuolated stratum of the "Keimhautblastem."
vit., vitellophag.
vt. g., ventral groove.

EXPLANATION OF PLATE I.

Unless otherwise stated all the figures on this and succeeding plates were drawn from eggs or embryos of Calligrapha multipunctata. Lines drawn through figures on this plate indicate where sections were made, and refer by number to more highly magnified illustrations on the following plates. The germ-cells (pole-cells) are represented by small rings. All are magnified 35 diameters.

Stage A. The pole-cell nuclei are protruding at posterior end. No blastoderm-cell nuclei have yet reached the surface.

Stage B. All the pole-cells (64) lie in a group outside of the egg at the posterior end.

Stage C. Ventral surface of egg. The lateral folds of the ventral plate have appeared.

Stage D. View of ventral surface. The group of pole-cells has been carried part way up on the ventral surface.

Stage E. Ventral view of egg. Pole-cells lie in posterior depression of ventral groove. First appearance of germ-band.

Stage F. Ventral groove narrower than in Stage E. Pole-cells partly covered by lateral folds.

Stage G. Lateral view of same egg as in Stage F. Dotted line shows depth or ventral groove. Pole-cells lie at entrance to pole-cell canal.

Stage H. Lateral view. Amnioserosal fold partly covers germ-band. Pole-cells lie at end of posterior amniotic cavity; a few have passed through the pole-cell canal.

Stage J. Germ-band, in lateral view, almost covered by amnioserosal fold. More pole-cells are inside of germ-band than in younger stage (H).

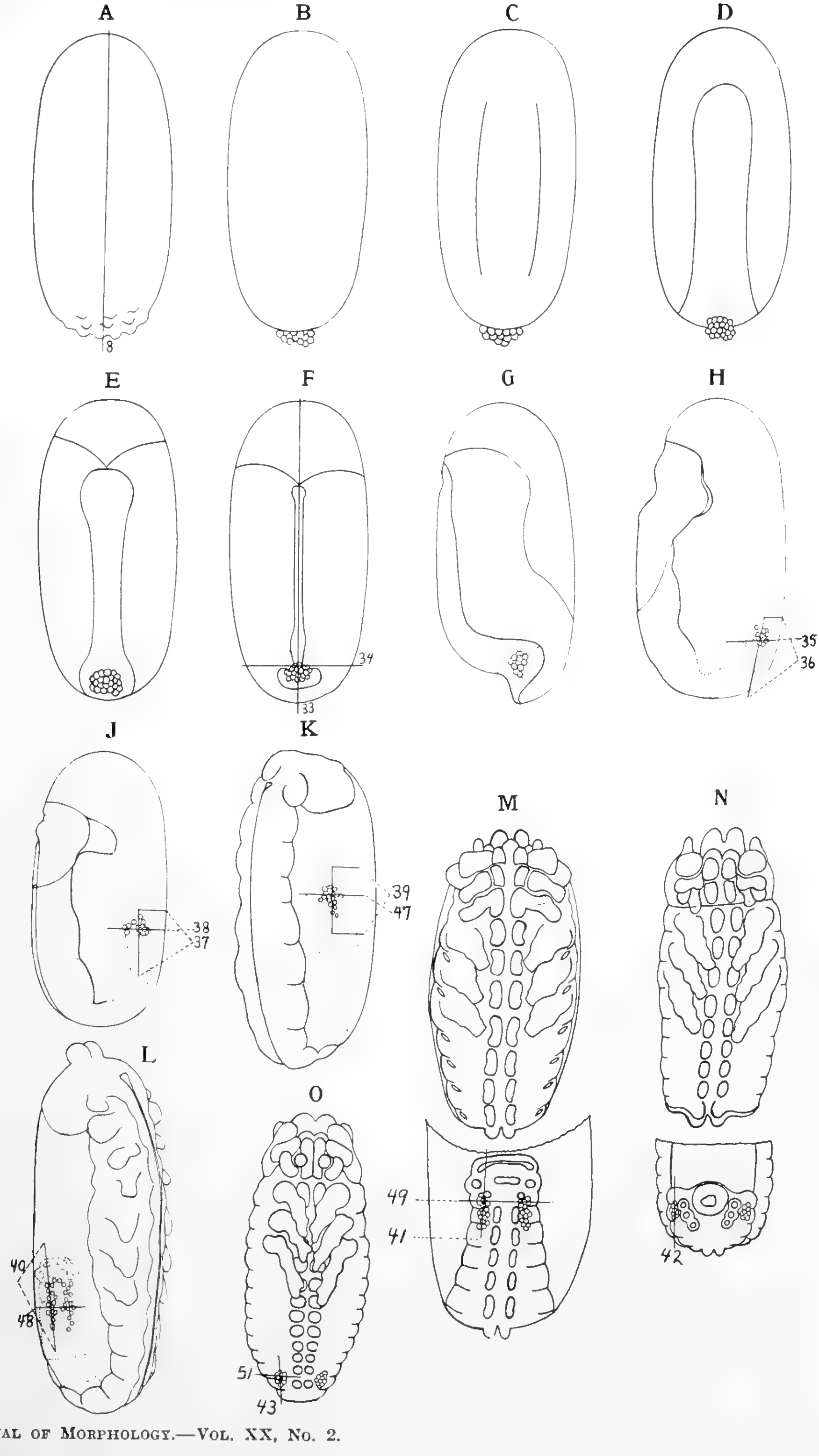
Stage K. View of lateral surface of segmented germ-band. Nearly all of the pole-cells are now inside the embryo.

Stage L. Lateral view after appearance of appendages. Tail-fold shorter than in preceding stage (K). Germ-cells have separated to form a group on either side of tail-fold in last two abdominal segments.

Stage M. Upper figure represents ventral surface of embryo; lower figure, the posterior portion on dorsal surface. Tail-fold is short and broad. Germ-cells are closer to one another than in Stage L.

Stage N. Upper figure represents ventral surface of embryo; lower figure, the tail-fold on the dorsal surface. Germ-cells form two definite compact germ-glands.

Stage O. Ventral view of embryo. End of tail-fold now coincident with posterior pole of egg. Germ-glands lie near ventral surface on either side of median line.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EXPLANATION OF PLATE II.

A number in brackets refers to the figure from which the cells have been enlarged.

FIG. 1. Surface view of posterior end of an egg 2½ hours after deposition. The pole-disc occupies about ⅛ of the entire area. Only those yolk-globules adjacent to the pole-disc are shown. a, circumference of egg indicated by single line. × 200.

FIGS. 2 to 4 represent portions of the posterior end of eggs in longitudinal section showing the arrangement of the pole-disc granules. × 850.

FIG. 2. Egg taken from the oviduct of Leptinotarsa. Granules of pole-disc are here very close together forming a broken strand.

FIG. 3. Egg 13 hours after laying. This egg contained about 133 nuclei. Granules of pole-disc suspended in the inner stratum of the "Keimhautblastem" forming a network.

FIG. 4. Egg 11 hours after laying. Pole-disc granules widely separated from one another.

FIGS. 5 to 11 represent longitudinal sections through the posterior end (except figure 8) of eggs, showing early stages in pole-cell formation. Granules of pole-disc represented by dots. × 60.

FIG. 5. One of the 4 pole-cell antecedents (a) is shown in this figure.

FIG. 6. Three of the nuclei (a) in this figure divide once giving rise to pole-cells.

FIG. 7. The three nuclei indicated at a will become pole-cells.

FIG. 8. Longitudinal section through an egg in Stage A. Seven pole-cell nuclei are protruding from the posterior end of the egg.

FIG. 9. Three of the six pole-cell nuclei represented in this figure have entirely separated from the egg.

FIG. 10. Blastoderm completed. Pole-cells form two layers.

FIG. 11. Pole-cells form an irregular group. a, pole-cell in anaphase of division.

FIGS. 12 to 15 represent surface views of the posterior end of eggs, showing successive stages in pole-cell formation. × 200.

FIG. 12. The four nuclei (a) under the center of the pole-disc will give rise to all of the pole-cells.

FIG. 13. Eight pairs of pole-cell nuclei may be recognized by their dark granules. Eleven of the blastoderm-cell nuclei (a) contain a few pole-disc granules. b, strand of cytoplasm connecting a pair of pole-cells.

FIG. 14. Thirty-four pole-cells are present, each containing granules from the pole-disc. One pole-cell (17) is in the late anaphase of mitosis.

FIG. 15. Sixty-three pole-cells are visible. The blastoderm is fully formed.

FIG. 16. Lateral surface view of the posterior end of egg in Stage B. Part of the pole-cells occupy an indentation in the end of the egg. The pseudo-blastodermic nuclei appear as a dark mass in the interior. × 200.

FIG. 17. Pole-cell in anaphase of mitosis. Enlarged from Fig. 14, position 17. The pole-disc granules have been equally distributed to the two ends of the cell. × 850.

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EXPLANATION OF PLATE III.

Fig. 18. Two nuclei which will give rise to four pole-cells; enlarged from Fig. 6, position 18. a, boundary between nucleus and cytoplasm; b, chromosomes partially arranged in equatorial plate. × 850.

Fig. 19. Two nuclei which will become pole-cells; enlarged from Fig. 7, position 19. a, pole-disc granules accumulated in compact mass. Compare pole-disc in Fig. 2. × 850.

Fig. 20. Two pole-cell nuclei (a and b) and one blastoderm cell nucleus (c); enlarged from Fig. 8, position 20. d, pole-disc granules which remain in "Keimhautblastem" after the pole-cells are protruded. × 850.

Fig. 21. One pole-cell nucleus enlarged from Fig. 9, position 21. The pole-disc granules entirely surround the nucleus. × 850.

Figs. 23 to 27 represent longitudinal sections of portions of the posterior end of eggs, showing the arrangement of the pole-cells, and the formation of the pseudoblastodermic nuclei and the pole-cell canal. × 200.

Fig. 23. Blastoderm ends abruptly where it encounters the pole-cells. a, pole-cell in anaphase of mitosis.

Fig. 24. A few blastoderm-nuclei have been pushed upward past the pole-cells into the yolk to form pseudoblastodermic nuclei.

Fig. 25. Pseudoblastodermic nuclei now form a funnel shaped syncytium just above the pole-cells.

Fig. 26. In this figure (Leptinotarsa) the pole-cells are larger and have larger nuclei than the blastoderm-cells. A few pseudoblastodermic nuclei (ps.bl.n.) are present.

Fig. 27. The pole-cells in C. lunata here show an arrangement similar to those in C. multipunctata (Fig. 24). a, one pole-cell in the last stage of division.

Fig. 28. A pole-cell and an adjacent blastoderm-cell enlarged from Fig. 25, (28). × 850.

Figs. 29 to 32 represent surface views of eggs showing how the group of pole-cells is carried into the posterior amniotic cavity. × 50.

Fig. 29. Ventral view (Stage E). A shortening of the germ-band has carried the pole-cells into the posterior depression of the ventral groove.

Fig. 30. Lateral view of same egg as Fig. 29. a, cephalic lobe of germ-band; b, invagination which will give rise to the stomodeum.

Fig. 31. View of posterior end of same egg as Fig. 29.

Fig. 32. Ventral view (Stage F). Pole-cells, partly covered by lateral folds, lie in the posterior depression of ventral groove (a). This depression is now the posterior amniotic cavity.

(*Continued on next page*)

(*Continued from preceding page*)

Fig. 33. Sagittal section through an egg similar to that shown in Fig. 32 (Stage F). The relations of pole-cells, pole-cell canal and pseudoblastodermic nuclei are here well illustrated. a, invagination which will give rise to stomodeum. × 60.

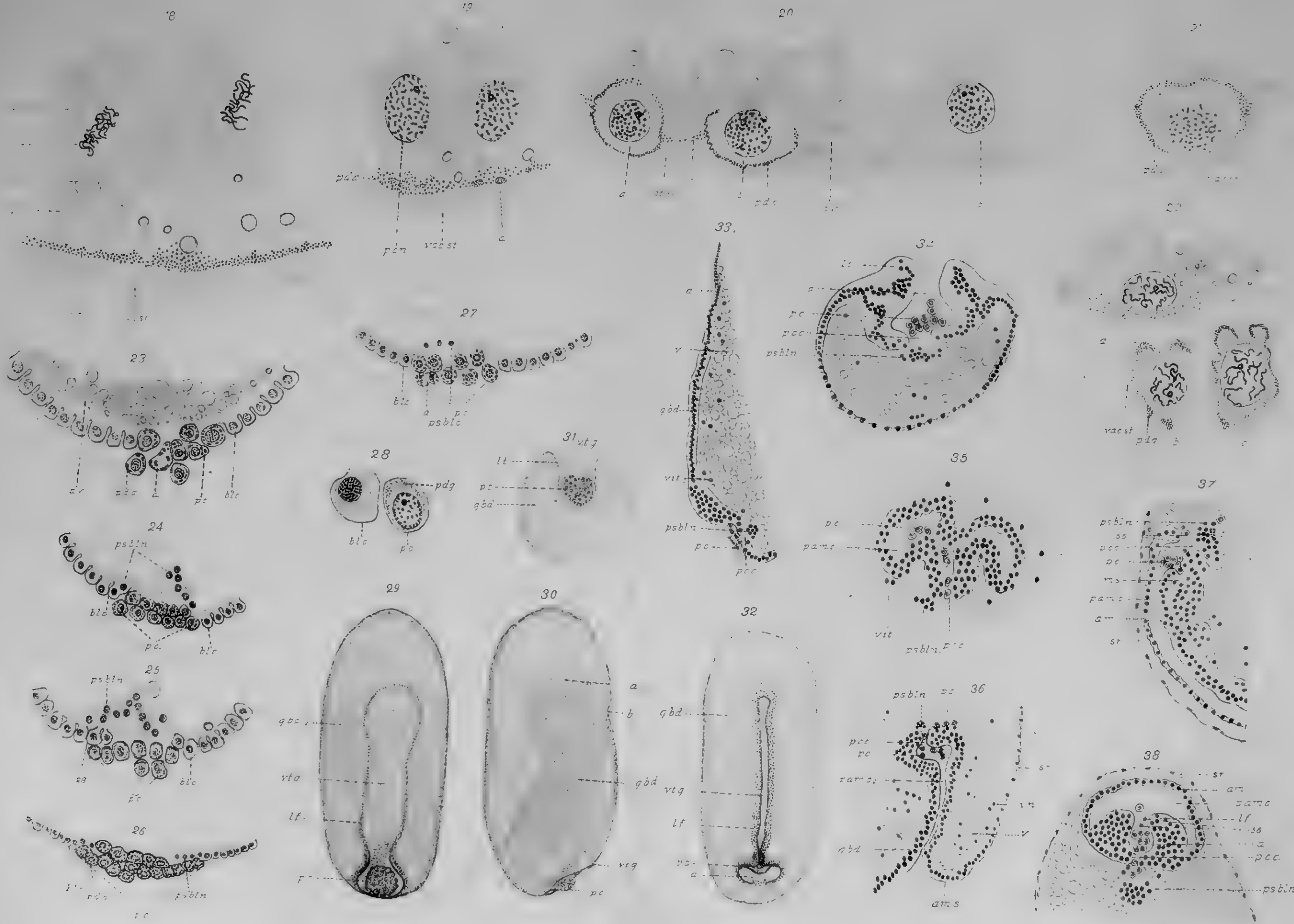
Fig. 34. Transverse section through posterior depression in ventral groove, Fig. 32 (Stage F). a, flask-shaped depression in ventral groove. × 105.

Fig. 35. Transverse section near the end of the tail-fold of an embryo of Leptinotarsa similar to Stage H, position 35. The pole-cells are creeping from the posterior amniotic cavity through the pole-cell canal. × 140.

Fig. 36. Sagittal section through the posterior end of an egg of Leptinotarsa like Stage H, position 36. One pole-cell is part way through the pole-cell canal. × 105.

Fig. 37. Sagittal section through the tail-fold of an embryo in Stage J, position 37. Mesoderm has appeared. A few pseudoblastodermic nuclei show signs of disintegration. × 105.

Fig. 38. Transverse section of tail-fold of embryo in Stage J, (38), showing passage of pole-cells through pole-cell canal. a, flask-shaped depression in ventral groove. × 105.



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EXPLANATION OF PLATE IV.

FIGS. 39 to 45. Sagittal sections through the tail-fold or posterior end of embryos, showing the migration of the germ-cells within the body, and their crowding together to form the germ-glands. Figs. 39 and 42, × 105; the others, × 140.

FIG. 39. Embryo to Stage K (39). Almost all of the pole-cells have penetrated into the embryo.

FIG. 40. Embryo in Stage L (40). a, two pole-cells lying in the much shortened pole-cell canal.

FIG. 41. Embryo in Stage M (41). The germ-cells are closer together than in a younger embryo (Fig. 40).

FIG. 42. Embryo in Stage N (42). The germ-cells form a distinct germ-gland with epithelium of mesoderm-cells.

FIG. 43. Embryo in Stage O (43). One of the germ-glands lies at the side of the median line near ventral surface of embryo.

FIG. 44. Embryo of 86 hours slightly older than Stage O. Germ-gland has been carried near dorsal surface by lateral growth of embryo around the yolk.

FIG. 45. Embryo of 105 hours (male). The germ-gland has become constricted, forming a dumb-bell-shaped structure.

FIG. 46. Sagittal section through germ-gland (female) of an embryo 105 hours old, showing the developing terminal filaments. × 320.

FIGS. 47 to 53. Transverse sections through the tail-fold or posterior end of embryos, showing the separation of the germ-cells into two germ-glands which are carried near the dorsal surface by the lateral growth of the embryo around the yolk. Fig. 47, × 105; all the others, × 140.

FIG. 47. Embryo in Stage K (47). Part of the pole-cells are still on their way through the pole-cell canal. (For sagittal section of this stage see Fig. 39.)

FIG. 48. Embryo in Stage L (48). Three pole-cells on each side of the tail-fold. Sagittal section in Fig. 40.

FIG. 49. Embryo in Stage M (49). Germ-cells lie close to coelomic cavity on either side of tail-fold. Sagittal section in Fig. 41.

FIG. 50. Embryo slightly older than Stage M. Germ-cells have acquired an epithelium of mesoderm-cells.

FIG. 51. Embryo in Stage O. Germ-gland on one side of median ventral line. Sagittal section in Fig. 43.

FIG. 52. Embryo 86 hours old. Germ-gland has been carried near dorsal surface. Sagittal section in Fig. 44.

FIG. 53. Embryo 105 hours old, showing germ-gland (female) near median dorsal line. The terminal filaments of the ovary are developing.

FIG. 54. Frontal section of an embryo in Stage O. × 140.

FIG. 55. A pole-cell and adjacent ectoderm-cell enlarged from Fig. 37 (55). × 850.

FIG. 56. Two pole-cells showing amoeboid processes, enlarged from Fig. 38 (56). × 850.

FIG. 57. Two pole-cells (a), two mesoderm-cells (b), and two ectoderm-cells (c), enlarged from Fig. 41 (57). × 850.

PLATE IV

R. W. HEGNER, DEL

THE DEVELOPMENT OF THERIDIUM, AN ARANEAD, UP TO THE STAGE OF REVERSION.

BY

THOS. H. MONTGOMERY, Jr.

With Eight Plates.

CONTENTS.

Introduction and Methods.

This paper presents an account of the morphological changes during the ontogeny of *Theridium tepidariorum* C. Koch, from the time of cleavage to the reversion of the embryo. The considerable gaps in our knowledge of this period of development of the spider and the conflicts of opinion of previous observers, particularly with regard to the formation of the germ layers, seemed to make this study worthy of the undertaking. In the comparisons drawn with the results of others I have limited myself almost entirely to the literature on araneads and have not considered that bearing on other arachnids, for I have had more interest in the ontogenetic processes than in the phylogenetic. Further, I have not treated the literature previous to the classical memoir of Claparède in 1862. Those papers published by Salensky and Morin in Russian I could not read, but have had to rely upon reviews, so that I may not have done full justice to these writers.

All my embryological material I have secured from spiders kept in captivity at the Woods Hole Marine Biological Laboratory during the summer of 1906, and have been able to get complete and accurately timed series of stages with comparative ease.

All the eggs within a given cocoon are of approximately the same age. The fixing fluid that proved the most satisfactory was that introduced by Carnoy: glacial acetic acid, absolute alcohol and chloroform in equal parts, with corrosive sublimate to excess. The cocoons were opened, the eggs dropped into this mixture and left in it from one to two hours. By this method nuclear structure and mitotic figures are generally excellently preserved, as well as the cytoplasmic structure. The yolk, on the other hand, becomes generally coagulated, sometimes into a homogeneous mass, also from the stage of about nineteen hours up to about the reversion a yolk extraovat is generally produced in the extraembryonic area. These disadvantages mattered little, however, for I have not given particular attention to the yolk changes. After the preservation the eggs can be cut with relative ease, for the yolk does not become brittle.

Stained mounts of whole eggs are most necessary, and beautifully clear preparations were made as follows: After fixation and hardening

the chorion was removed with needles, the egg then overstained in Delafield's haematoxyline. Destaining was then carried out in acid alcohol until the egg became a pale pink color, with all stain removed from the yolk and cytoplasm; xylol was used for clearing and Canada balsam for mounting. When the ectoblast has secreted a cuticula, as at the time of reversion, it is necessary either to stain longer or else to remove a portion of the embryo with a scalpel, so as to allow satisfactory penetration of the stain. For sectioning the chorion was removed, the embryo stained in eosin in the absolute alcohol used for dehydration (so as to make it clearly discernible in the paraffine), and cleared in xylol. Serial sections were cut 7 micra thick, and these stained with either Delafield's haemotoxyline or iron haemotoxyline followed by eosin. The embryos were so imbedded in rows that several could be cut at once, whereby sections in all desirable planes could be quickly secured. I have mentioned these methods somewhat in detail, for eggs of spiders have generally been considered difficult to treat.

Theridium tepidariorum is an exceptionally favorable form for study, because timed stages are secured with facility, the females being easily kept, and the eggs are conveniently small.

1. Observations.

1. *The Cleavage Up to Gastrulation.*

The first cleavage spindle is found at from 3 to 4 hours after oviposition; the two-cell stage at from 4 to 5½ hours, and the four-cell stage at from 6 to 7 hours.

In Pl. I, Fig. 1, is seen the anaphase of the first cleavage, the commencement of the two-cell stage. The daughter nuclei lie each adcentral within a mass of cytoplasm, and these central cytoplasmic masses are connected with the thin peripheral layer (blastema) by a network of delicate strands coursing between the yolk columns. In the figure indicated only a few of the coarser of these strands are shown, for most of them are too delicate to be shown at this scale of magnification. My account of the fertilization (1907) explained how this protoplasmic arrangement comes about; how the young oöcyte contains dense cytoplasm from the center to the periphery, and how

by the deposition of the yolk the cytoplasm becomes forced into those positions not occupied by the yolk. Accordingly, in the stage of Fig. 1 cytoplasm is probably present everywhere between yolk spheres, though for the most part in minimal amount. The peripheral blastema is present throughout cleavage and does not become marked into cell territories until all the cleavage cells have moved into it; the areas it exhibits on surface views are simply impressions due to the underlying yolk spherules.

The early four-cell stage is presented in Fig. 2, with the nuclei a little further apart from the centre of the egg. Fig. 3 shows an eight-cell stage (7½ hours); this drawing is a combination from a series of sections, and only the superficial blastema (*Cyt*), the eight cleavage cells and certain supernumerary sperm nuclei (*Sp*) are shown; those cells in the upper half of the egg are shaded, those of the lower hemisphere shown in profile only. Fig. 4 represents a portion of one section of the same egg. Polyspermy is frequent, as I described in my account of the fertilization, though only one sperm nucleus passes to the center of the egg to become a male pronucleus, the others always remaining at the periphery. Later than the stage when the cleavage nuclei have reached the surface I have been unable to distinguish such accessory sperm nuclei with certainty, and cannot tell whether they take part in the formation of the blastoderm.

The sixteen-cell stage occurs in eggs aged from 8 to 10½ hours after oviposition. All the nuclei of such a stage, each in the metaphase of mitosis, are shown in Fig. 5, a reconstruction from a series of sections (like Fig. 3). The cleavage cells have moved still nearer the surface, as shown best in Fig. 6, a portion of a single section. I have not attempted to draw the separate yolk globules from this stage on, for after the action of the fixative employed the yolk generally coagulates into a more or less homogeneous mass. Still another action of this fixative is to be seen from this stage on, the formation of a central fluid cavity (*Cav.*, Fig. 6), but since this is found at only a particular stage it is probably not an artifact. But, though, after this method of preservation the yolk often becomes greatly changed from the natural condition, the cellular structures are shown with the greater clearness and fidelity. As the cleavage progresses the cells on near-

ing the egg surface draw with them the intravitellar cytoplasmic network.

The thirty-two-cell stage (11 hours) is represented in Fig. 7, a reconstruction from serial sections (made like Figs. 3 and 5); some of the sections were broken and only thirty nuclei could be found. None of the cleavage cells have yet reached the egg surface. Inequality in the rate of cell division is commencing, for while a few nuclei are in the rest stage most of them are in mitosis.

A stage of 12 hours with from sixty to seventy cells is shown in Fig. 8, a surface view of one hemisphere of the egg. Few of the cells are quite at the surface, but all of them close to it; this is the earliest cleavage stage at which the cleavage cells can be distinctly seen on whole mounts. The heavy line, *Cav. B,* marks the boundary of the central fluid cavity. The cells, still without separating walls, are connected by delicate branching processes.

The next stage is that of the early blastoderm with all the cleavage cells at the surface. The one figured is of the age of 11½ hours, but from this time on the age is no true gauge of the stage. A surface view of one hemisphere is drawn in Fig. 10, and a segment of a single section in Fig. 9. The cells are fairly evenly distributed on the periphery, not noticeably more numerous on any one pole than at another, and number about 140. It is to be noted that no cleavage cells remain within the yolk.

The movement of cells toward the surface is a peripheral movement of the whole intravitellar cytoplasm, so that when the cell bodies have merged with the blastema cytoplasmic threads penetrate for only a short distance into the yolk (Fig. 9).

Fig. 11 exhibits on surface view a hemisphere of an egg of 14 hours, a stage of about 300 cells. The cell bodies are beginning to project slightly above the surface of the egg.

The next stage shows the beginning of the segregation of the ventral germ disc or embryonic region (ventral plate), which is characterized by a greater number of cells, while the extraembryonic (dorsal) area possesses fewer cells. Pl. II, Fig. 12, is a lateral surface view showing 173 cells on this side, the total number of cells being about 250; and Fig. 13 is a section of the germ disc. Now

for the first time appear distinct cell membranes, for heretofore the cleavage cells lacked them; these membranes arise by the cells coming into mutual contact coincident with the shortening of their fibers. Consequently, cell membranes are produced first at the ventral pole, gradually they develop upon all cells of the germ disc as these cells multiply and become more crowded. But the extraembryonic cells remain membraneless until well past the gastrulation period, thus retaining the character of early cleavage cells.

The establishment of the germ disc seems due to two factors: (1) Mainly to a more rapid multiplication of cells at the ventral pole, as shown by their becoming progressively smaller there; and (2), to less extent, by movement of cells toward that pole, shown by the cells becoming less numerous on the dorsal hemisphere.

2. *The Earlier Part of the Gastrulation.*

The beginnings of the blastopore are found in eggs aged from 21 to 30 hours, when the blastoderm contains from 400 to 500 cells. On unstained germ discs viewed in alcohol there appears an eccentric whitish spot (*Cum. A,* Pl. II, Fig. 18), and this spot later includes a small pit (Fig. 23); on stained preparations this shows darker than the remainder of the germ disc (Figs. 15, 22). This spot is the first region of cell immigration, whereas the remainder of the germ disc is one-layered. It is always eccentric, and the margin of the germ disc nearest to it will become the posterior or caudal region of the embryo; therefore, with its appearance there can be distinguished for the first time anterior and posterior, as well as right and left. For convenience this may be called the "anterior cumulus" (*Cum. A* of the figures), since a second or "posterior cumulus" will later arise behind it.

A section of the earliest stage of the anterior cumulus of an egg of 21 hours is shown in Pl. II, Fig. 14; two cells have moved beneath the blastoderm in consequence of vertical mitosis. In an egg of 24 hours about eight cells have invaginated; Fig. 15 is a ventral view of the germ disc with about 417 cells (but its total number of cells is somewhat greater, for a portion of the disc lies on the other surface of the egg); Figs. 16 and 17 exhibit the anterior cumulus

on cross sections. From the start this cumulus shows a pit-like depression, not a linear groove, the gastrocoel (*Gast.,* Fig. 16); this deepens as the gastrulation proceeds. Fig. 19 is a cross section of a stage in which twelve to thirteen cells have pushed in; Figs. 20 and 21, of one with about twenty-five invaginated cells, and Figs. 22-25 of a still later stage, when the germ disc contains more than 500 cells. This gastrulation process is a double one: (1) By vertical mitoses of cells of the region of the cumulus, and (2) by inrolling of cells, else a gastrocoel could not be formed. The pressure of the yolk causes the gastrocoel to remain a rather shallow pit.

All the cells of the germ disc retain short processes penetrating into the yolk, these being the last remnants of the former intravitellar mesh. But those cells that invaginate develop longer processes and become more irregular in form (Figs. 17, 19, 21, 24, 25). The innermost of the invaginated cells begin to separate from the others and, as the earliest yolk cells, vitellocytes, to wander into the yolk (Figs. 21, 24, 25). These cells are larger than those on the surface of the germ disc, they are assimilating yolk more rapidly, and for the most part possess also larger nuclei.

At its periphery the germ disc is not sharply delimited from the extraembryonic area (Figs. 15, 22); as its cells increase by mitosis they become more crowded together, whereby their cell membranes appear more distinct, their intercellular processes shorter and thicker, and they come to project more above the surface of the yolk. Where these cells are most numerous they have completely merged with the blastema, consequently this blastema remains distinct from cell masses only at the periphery of the germ disc and in the extraembryonic area (Fig. 16).

The extraembryonic cells are not dividing, are widely separated from each other, membraneless and much branched; they stain less deeply than those of the germ disc. When a yolk extraovat is produced by the action of the fixative it is formed in the extraembryonic region.

3. *The Later Part of the Gastrulation.*

The stages now to be described are found in eggs from 30 to 55 hours, with from 1000 to 1500 superficial cells on the germ disc.

There is to be noted particularly the origin of the posterior cumulus, the rapid proliferation of vitellocytes and the segregation of the early mesentoblast.

On unstained germ discs examined in alcohol is to be found behind the anterior cumulus a second, smaller prominence, the posterior cumulus (*Cum. P*, Pl. II, Fig. 29; Pl. III, 32, 33; Pl. IV, 44). This is variable in position, placed sometimes at the margin of the germ disc, sometimes nearer the anterior cumulus (an extreme case of which is shown in Fig. 32). Both cumuli are shown on profile on a stained germ disc in Fig. 26, Pl. II. The two cumuli, at first generally separated, become later connected by vitellocytes moving between them beneath the germ disc; this is well shown on a surface view in Fig. 34, Pl. III, where the shaded portion marks the band of vitellocytes. A line connecting the two cumuli marks the later mid axis of the embryo, though, as we have seen, this could be foretold from the eccentric position of the anterior cumulus alone.

The posterior cumulus differs from the anterior, besides its later development, in being a prominence from the start, in forming no gastrocoel, and in producing only vitellocytes. But since it is a region of inner cell proliferation it may well be considered one part of a blastopore, the other part of which would be the anterior cumulus; probably the blastopore was phyletically first a longitudinal groove, the middle portion of which later disappeared. The earliest stage found of the posterior cumulus, one of 30½ hours, is shown on surface view in Fig. 26, Pl. II, and on median section in Fig. 27; it is then composed of a few large cells ingesting yolk. Later stages of it are illustrated in Figs. 30a, 36, 41a, Pl. III; 42a, 42b, Pl. IV, on median section, and in Figs. 35, Pl. III, and 43c, Pl. IV, on cross section. Even in the late stage of Fig. 43c there are only about twelve vitellocytes at the posterior cumulus, though these are unusually large; and Figs. 42a and 43c indicate that such cells are produced not only by invagination, but also by direct metamorphosis of superficial cells of the germ disc at that point. It is by the presence of this group of large yolk cells that one is enabled to identify the position of the posterior cumulus with the posterior end of the embryo of the succeeding protozonite stage.

Besides fixing the axes of the embryonic region the development of the posterior cumulus determines an important boundary. Just anterior to the posterior cumulus the cells of the germ disc are thinner than elsewhere (*Th. Ab,* Figs. 41a, Pl. III; 42a, Pl. IV); this will become the boundary of the cephalothorax and abdomen.

We may next consider the more important processes that are progressing at the anterior cumulus. This has become larger and more irregular in outline (*Cum. A,* Fig. 29, Pl. II; 32, 33, Pl. III; 44, Pl. IV), and is slightly elevated above the surface of the germ disc (Fig. 26, Pl. II). It maintains its pitlike gastrocoel (*Gast.,* Figs. 27, 28, Pl. II; 37b, 38A, B, Pl. III), that closes at the stage illustrated by Figs. 40A, B, 41A, C, Pl. III. It is still somewhat variable in position, though always behind the center of the germ disc. At the stage of 30½ hours it is shown on median section in Figs. 27 and 28, Pl. II; the invaginated cells have increased in number and size, and those in contact with the yolk have become greatly branched and coarsely vacuolar with ingested yolk particles. These large cells are vitellocytes, and they undergo a continuous emigration from their point of origin, which is in part a movement into the yolk, but to greater extent a passage from the periphery of the cumulus outward between the yolk and the germ disc; this is what causes the outline of the cumulus to become larger and more irregular. At a little later stage this wandering becomes more pronounced, as shown in the middle of Fig. 31, Pl. III (a cross section through the anterior edge of the anterior cumulus), and Fig. 30a (where only a lateral edge of this cumulus is cut).

A more advanced stage of the anterior cumulus, 37½ hours, is represented in Figs. 34-39, Pl. III. Fig. 34 is a ventral surface view of a germ disc containing 1222 superficial cells; the shaded region marks the area where vitellocytes lie, in a broad band extending from a little anterior to the centre of the germ disc back to the posterior cumulus (*Cum. P*); the two cumuli have become interconnected by vitellocytes arising mainly from the anterior one. Fig. 38a, an oblique longitudinal section of the whole germ disc, and Fig. 38b, an enlarged drawing of the anterior cumulus alone, show the gastrocoel to be a pit bounded immediately by a group of large unbranched

cells, around which are the large vitellocytes (*Vit. C.*). But a more interesting change is found in the egg, of which two transverse sections are depicted in Figs. 37a and 37b. Fig. 37b is through the gastrocoel. Fig. 37a, a few sections distant from the preceding, shows just beneath the superficial cells of the germ disc a compact group of from six to eight rounded cells (*Mes. E.*, only four of them visible in this section), which resemble the outer ectoblast cells. These are evidently the earliest cells of the mesentoblast, because they have the same situation and appearance as cells which later can be recognized with certainty as mesentoblast. But whence they originated I have not been able to determine, the question being whether they are direct derivatives of the ectoblast or from some particular invaginated cell of the anterior cumulus. The latter view would seem the more probable, judging from their position within the anterior cumulus.

At the next stage seen, one of about 49 hours, both vitellocytes and mesentoblastic cells have increased in number and come to occupy a wider area. Fig. 40a, Pl. III, shows all the superficial nuclei of the germ disc on ventral view, and they number 1441; at the posterior cumulus (*Cum. P.*) the nuclei are larger because there the vitellocytes reach the surface. Fig. 40b is a drawing of the same egg, but at a deeper focus, exhibiting only the nuclei of vitellocytes beneath the superficial cells; this figure demonstrates that the vitellocytes are now scattered beneath the whole of the germ disc; this figure does not reproduce all of the vitellocytes, but only those whose nuclei could by their superior size be readily distinguished from the nuclei of the surface cells. Sections further illustrate this migration of vitellocytes; thus Figs. 41a, Pl. III, and 42a, Pl. IV, show their position on median sections; Figs. 41b and 41c, Pl. III, on longitudinal sections of the middle of the germ disc; Fig. 43a, Pl. IV, on transverse section of the disc anterior to the anterior cumulus; and Fig. 43b on cross section lateral to this cumulus. Accordingly, in this stage the large, markedly branched and richly vacuolated vitellocytes are still most abundant in the vicinity of the anterior cumulus, but many of them have emigrated thence, some into the yolk, a greater number in all directions beneath the germ disc, while there remains at the

posterior cumulus the group of them that formed there. This movement of vitellocytes from their point of origin comes to lower the elevation of the anterior cumulus and to cause it to become flush with the surface of the germ disc.

At the same time mesentoblast cells are scattered beneath the germ disc except at its posterior pole (for they do not appear to arise there). These are polygonal and relatively small, placed between the outer cell layer and the yolk or yolk cells; they are lettered *Mes. E.* in Figs. 41bc, Pl. III, and 42b, 43a and 43b, Pl. IV. They do not compose a continuous layer and are only one cell deep except at one point, where they are two deep (Fig. 41c, Pl. III). I have searched carefully but in vain to find indications that these mesentoblast cells develop *in situ* from the overlying ectoblast; all the mitotic spindles of the ectoblast seem to be placed horizontally and none vertically at this stage. Therefore, it is probable that the mesentoblastic elements of this stage are emigrated descendants of that group of six to eight cells of the previous stage (*Mes. E.,* Fig. 37a, Pl. III) which formed part of the anterior cumulus. They seem to have wandered from a single point of origin, just as the vitellocytes have done. Definitive entoblast and mesoblast will later form from this mesentoblast, as will be described in due time. There is closure of the gastrocoel at this period, one of several indications that gastrulation is ending and consequently cellular invagination, and there is no indication at any later stage that either mesoblast or entoblast forms from the outer cell layer; therefore, the latter from now on may be termed ectoblast. The ectoblast cells are becoming higher than wide. Thus, the germ disc is at many points two-layered, consisting of outer ectoblast and inner mesentoblast, both placed outside of the vitellocytes.

Still another process is under way, namely, formation of vitellocytes from the anterior and lateral margins of the germ disc, an origin quite independent of the centers of formation represented by the two cumuli. Their formation from the anterior margin of the germ disc is shown in Figs. 30a, b, 38a, 41a, Pl. III, and from the lateral margin in Figs. 31 (an unusually pronounced case) and 39. Whether this is effected by vertical mitoses or by inrolling of the edge of the germ disc I have not positively determined, but there are indi-

cations of the former process (note the position of the mitotic spindle at the left edge of Fig. 31). This process results in a heightening of the margins of the germ disc, which is well exhibited on alcoholic surface views (Figs. 29, Pl. II; 33, Pl. III; 44, Pl. IV). The vitellocytes produced thereby do not differ in appearance from the others. Branched vitellocytes rarely show signs of division, and it is probable that those which have become large and ramified do not divide; they do not wander beneath the extraembryonic blastoderm.

The germ disc has not increased perceptibly in extent, but has become sharply delimited from the extraembryonic area (Fig. 34, Pl. III). Its cells are closely apposed and have lost their intercellular branches. In the extraembryonic region the blastoderm still consists of membraneless branched cells.

4. *Stage of the Protozonites.*

This stage is evidently of short duration, for I found it in only two lots of eggs, of the age of 60 hours. Fig. 45, Pl. IV, exhibits the earliest condition seen, one with four rather indistinct protozonites, and Figs. 46 and 47 (lateral and ventral views, respectively) with five protozonites. The germ disc has changed from a circular to an ovoid outline, with one end broader than the other; the broader end marks the beginning of the cephalic lobe, and the narrower, the caudal. There are no longer projecting cumuli, but on median section the caudal end (*Caud.*, Fig. 48) is seen to correspond in position with the earlier posterior cumulus by the continuance of the group of large vitellocytes at that point (*Vit. C.*). The five protozonites of Figs. 46 and 47 appear on stained whole mounts darker than the intermediate regions because they are thicker, show no signs of appendages and represent the beginnings of the segments of the pedipalps and the four pairs of legs, while the protozonites of Fig. 45 represent the segments of the pedipalps and the three anterior pairs of legs. The boundary between cephalothorax and abdomen is that point where the ectoblastic cells are somewhat flattened (*Th. Ab.*, Figs. 48, 50b); previously this region had lain just anterior to the posterior cumulus (Figs. 41a, Pl. III; 42a, Pl. IV). On comparison of Fig. 41a with Fig. 48 it follows that the elongation of the germ disc pro-

ducing the abdomen is due to a rapid growth between this thin region of the ectoblast and the posterior edge of the germ disc (region of the posterior cumulus), and not to growth cephalad from the posterior end of the germ disc. The abdominal region is therefore extending teloblastically, by cell multiplication, in its anterior portion. It results that the thin region of the ectoblast (*Th. Ab.*, Fig. 48) is rapidly separating from the group of vitellocytes at the posterior end (caudal lobe, *Caud.*). It will be noted that the thoracal segments develop *in situ* and almost if not quite simultaneously, teloblastic growth occurring only in the abdomen. The extension of the embryo around the yolk, shown in Fig. 47, is due mainly to growth of the abdominal region, dorsal extension of the head lobe being much less in amount.

The middle cell layer, that between ectoblast and vitellocytes, is still one cell deep (Fig. 48), and its cells are smaller than those of the ectoblast. This layer within the cephalothoracal region is true mesoblast (*Mes.*, Figs. 50 a-c), for there is at no time any indication that entoblast arises within the cephalothorax; it is only much later than the stage of reversion that entoblast enters the cephalothorax, and then by growth of the midgut from the abdomen into the posterior part of the thorax. This cephalothoracal mesoblast is segmented, each of its transverse masses confluent with and, indeed, occasioning a protozonite, while it is absent between protozonites; Fig. 48 shows this condition on median section of the whole embryo, and Fig. 49b on transverse section of one-half of a protozonite. This segmented condition of the mesoblast is a secondary one, for in the preceding stages it showed no such distribution. In the head region (Fig. 50a) there is a layer of mesoblast, as in the thorax, and here also it is segmented, for it shows a division into a more anterior rostral mesoblast (*R. Mes.*) and a more posterior cheliceral mesoblast (*Chel. Mes.*); this is important as indicating that in this early stage there are two mesoblastic sacs within the cephalic lobe, one anterior to and distinct from the cheliceral segment.

In the abdominal region there is a single unsegmented layer of cells beneath the ectoblast, extending from the thoraco-abdominal boundary (*Th. Ab.*, Fig. 50b) not quite to the posterior margin (*G. B.*) of the germ disc. This layer is mesentoblast, as its later history shows.

The ectoblast consists of cells that are mainly columnar, and in some places it is becoming two cell layers deep (Figs. 49b, 50a-c).

The vitellocytes have become relatively enormous (*Vit. C.*, Figs. 48, 49b, 50a-c), and most of them lie in the more superficial portion of the yolk, few having reached its center. For the first time the extraembryonic blastoderm is beginning to proliferate vitellocytes by direct metamorphosis of some of its cells (Fig. 49a). The extra-embryonic blastodermic cells are also increasing in number.

5. *Stage of One to Two Abdominal Segments.*

Fig. 51, Pl. IV, is an oblique latero-ventral view of the stage, 60¼ hours, immediately following that of the protozonites. It shows the pedipalpal segment (*Ped.*) and the segments of the legs (*L.* 1-*L.* 4), all with the first traces of limb buds. Fig. 52, an embryo of ca. 62 hours, illustrates a lateral view of the later stage with six pairs of cephalothoracal appendages (the cheliceral segment, *Chel.*, now separated from the head lobe, *Ceph.*), and the appearance of the first abdominal segment (*Ab.* 1). Then Fig. 53 illustrates a still later stage, where there are two abdominal segments (*Ab.* 1, *Ab.* 2) and a trace of a third. On comparing Fig. 52 with Fig. 53 it will be seen that it is the abdominal region that is lengthening most rapidly, which results in the caudal lobe (*Caud.*) pushing dorso-cephalad until it nearly meets the head lobe. The nuclei of the extraembryonic region are marked by stippling.

The stage of Fig. 53 merits a more detailed description.

The mesoblast of the cephalothorax is now arranged in the form of a series of paired pouches, discontinuous longitudinally and transversely; this segregation is the mechanical cause of the limb buds. In the chelicera (*Chel.*, Fig. 56c, Pl. V) and the fourth leg pair (*L.* 4, Fig. 56a) it is only one layer deep, for in these segments it has developed more slowly than in the others; but beneath the other thoracal appendages it is two cells deep (Figs. 55, 56d). When this two-layered condition has been reached the layer next the ectoblast may be called somatic mesoblast (*So. Mes.*, Fig. 55), and the other layer, splanchnic mesoblast (*Sp. Mes.*). The way in which the splanchnic layer becomes separated from the somatic is shown in Fig. 56d, indicating

its formation by inrolling of the edge of the somatic rather than by vertical mitosis or delamination. At first the two layers are closely apposed, but they later partially separate to produce the coelom (*Coel.*, Fig. 55). At no time do yolk globules enter any of the coelomic cavities.

In the cephalic region the mesoblast of the cheliceral segment (*Chel. Mes.*, Fig. 56c) is separated from the more anterior rostral mesoblast (*R. Mes.*).

In the abdominal segments (*Ab.* 1, *Ab.* 2, Fig. 56a) the mesentoblast is transversely and longitudinally segmented into paired bands, but is still one-layered. Posterior to these segments, within the caudal lobe proper (Fig. 56b), the mesentoblast (*Mes. E.*) is a continuous layer. On transverse section of the caudal lobe are found at occasional intervals in the midline masses of small branched cells (*G. C. ?*, Fig. 54, compare also Fig. 56b); these are much smaller and stain more deeply than vitellocytes, and may represent either genital cells or early definitive entoblast.

Vitellocytes are still forming by metamorphosis of cells of the extraembryonic blastoderm.

6. *Stage of Three to Five Abdominal Segments* (73 *to* 75 *Hours*).

Fig. 57, Pl. V, shows a stage with three abdominal segments, Fig. 58 one with four, and Fig. 59 one with five. The abdomen has increased in length until the caudal lobe meets the head lobe on the dorso-anterior surface of the yolk. The posterior unsegmented portion of the abdomen is the caudal lobe of the authors, comparable with the telson of other animals having teloblastic growth. While the abdominal segments still lack limb buds the appendages of the cephalothorax have become short and blunt cylinders directed caudad. The head lobe (*Ceph.*, Fig. 59) shows as yet no particular organ regions, but extending from it backwards along the thorax is a light median line, the ventral sulcus (*Sul. v.*), which marks the region where the ectoblast is thinnest and from which the mesoblast sacs have withdrawn laterally.

The first traces of the central nervous system now appear, paired thickenings of the ectoblast mesial from the appendages in that

region from the pedipalpal to the fourth ambulatory segment. In such ganglionic thickenings (*Gang.*, Fig. 62) the ectoblast is two-layered, while in the ventral sulcus (*Sul. v.*) between them it has become thinner; therefore, cells have probably moved away from that ventral midline to aid in the production of the ganglia. Such ganglia cannot yet be distinctly seen upon surface views, and those of the chelicera are not yet differentiated.

In the head lobe the posterior cheliceral mesoblast (*Chel. Mes.*, Fig. 60b) is distinct from the more anterior rostral (*R. Mes.*, Fig. 60a). A slight elevation on the surface of the head lobe seems to indicate the first appearance of the cerebral ridges. In the thorax each appendage has its mesoblast sac, but these sacs extend neither mesial nor lateral of the appendages (Fig. 62, transverse section).

The abdomen where it is segmented exhibits its mesentoblast in segmented two-layered masses, but in the caudal lobe in a single layer. Each segment has a right and left mesentoblastic mass separated in the midline from each other (Fig. 61). Where this cell mass is two layers deep the outer layer is somatic mesoblast. (*So. Mes.*), while the inner is still mesentoblast (*Mes. E.*). At various points in the median axis of the abdomen are groups of small cells (*G. C.* ?, Fig. 61), which had been remarked in the preceding stage.

Fig. 61 shows that vitellocytes are still forming from the extraembryonic blastoderm.

7. *Stage of the Early Abdominal Appendages* (86 *Hours*).

The external characteristics of embryos of this period are shown in Figs. 63-66, Pl. V. The caudal lobe (*Caud.*) has reached the head lobe, the ventral sulcus (*Sul. v.*) is widening and extends posteriorly to the seventh abdominal segment (Fig. 66), which is one factor in the lateral expansion of the body, and in consequence the extraembryonic area has decreased in amount (the stippling represents the nuclei of this area in their actual number). The lateral view, Fig. 63, shows how the embryonic region has encroached upon the extraembryonic as compared with the preceding stage, Fig. 57. Of the cephalothoracal appendages the cheliceral (*Chel.*, Figs 63, 65) are the shortest, while the others (*L.* 1-*L.* 4) have become three-

jointed. The abdomen possesses eight segments (*Ab.* 1-*Ab.* 8, Figs. 63, 66) anterior to the caudal lobe (*Caud.*), the full number that it will have in this species; and of these the second to the fifth inclusive bear each a pair of limb buds (*Ab. 2b-Ab. 5b*). For each of the thoracal segments (Fig. 65) and to each of the abdominal except the eighth (Figs. 63, 66) there is a pair of nerve ganglia.

On the head lobe appears the stomodaeum (*Sto.,* Figs. 64, 65), a shallow pit with somewhat turgid lips (*Sto. L.*). It is an ectoblastic invagination shown on median section in Fig. 67b, Pl. VI, and on cross section in Fig. 68b. Just at its anterior border is a pair of small, basally contiguous prominences, the rostral appendages (*Ros.,* Figs. 64, 65, Pl. V) ; later these will fuse to form the rostrum. They are shown on longitudinal section in Fig. 67b, Pl. VI, and on transverse section in Figs. 68a and 68d. Beneath these appendages lie the rostral mesoblast sacs, which occupy more than the anterior half of the head lobe, meet in the midline anterior to the ventral sulcus, and are continued in the lateral lips of the stomodaeum. The upper portion of Fig. 68d shows how these sacs extend laterally almost as far as the head lobe itself; Fig. 67b shows that they extend mesially back to the posterior border of the stomodaeum; and Fig. 67a shows one rostral sac on longitudinal section in the plane of a cheliceron (*Chel.*), this demonstrating how much more extensive the rostral sacs are than the cheliceral. Each rostral sac now consists of somatic and splanchnic layers, and these layers separate from each other to form coelomic spaces beneath the rostral appendages (*Ros.*, Figs. 67b, 68a and 68d) at the lateral margins of the head lobe (*R. Coe l.,* Figs. 68c-f), and beneath the cerebral ridges (*Ce. R.,* Figs. 67a, 68f). The only prominences of the head lobe anterior to the chelicera that can be properly considered prestomial appendages are these rostral tubercles; and they may be rightly adjudged cephalic appendages to which belong the rostral mesoblast sacs, and the ganglia of which would be the cerebral. Though they develop later than the chelicera it will be recalled that the chelicera arise later than the pedipalps and the legs, and the rostral mesoblast sacs develop simultaneously with the cheliceral. Fusion of these rostral appendages with the lips of the stomodaeum follows later, the two are

separate in origin, and the stomodaeal lips (*Sto. L.,* Fig. 67b) are simply ectoblastic thickenings into which extends the rostral mesoblast.

Other differentiations of the head lobe are the following: A short distance anterior to the rostral appendages the head lobe is mesially broadly indented, probably by its lateral borders growing more rapidly than its median, this constituting the anterior sulcus (*Sul. A.,* Fig. 64, Pl. V). Behind this is the stomodaeum (*Sto.*) and behind that the ventral sulcus (*Sul. v.*), so that the head lobe is nearly completely divided into right and left halves. The antero-median margin of each half is somewhat elevated and thickened, and each such transverse prominence, which may be called a cerebral ridge (*Ce. R.,* Figs. 63, 64) is bordered posteriorly by a groove, the fovea (*Fov.*). A longitudinal section through a cerebral ridge and fovea is given in Fig. 67a, Pl. VI. Fig. 68f shows a transverse section of the two cerebral ridges; in the midline lies the apex of the caudal lobe (*Caud.*) and immediately above that the extraembryonic blastoderm (*Ex.,* this being the plane of the anterior sulcus); right and left of this sulcus are the halves of the head lobe, the mesial portions of which are the cerebral ridges (*Ce. R.*). These ridges cannot be considered separate appendages because they do not possess peculiar mesoblast, but are bordered by the rostral mesoblast. Lateral from and on a line with the fovea of each side is a transversely elongated pit which may be termed the antero-lateral vesicle (*A. L. V.,* Fig. 63, Pl. V); this is difficult to find on surface views, but on transverse sections (*A. L. V.,* Figs. 68c, d, Pl. VI) each is found to be an ectoblastic groove. In most of the head region the ectoblast is several layers deep.

The chelicera (*Chel.,* Figs. 64, 65, Pl. V) are at the posterior border of the head lobe, as are their ganglia (*Chel. G.*), far behind the stomodaeum (*Sto.*). Their mesoblast sacs (Fig. 67a, Pl. VI) are separated from and much smaller than the rostral sacs.

Each thoracal limb behind the chelicera possesses a distinct coelom, bounded by somatic mesoblast extending into the limb and a splanchnic layer upon the yolk (Figs. 68d, e, 70). These mesoblast sacs do not as yet extend laterad of the limbs, but have grown some distance

mesiad beneath the ganglia (Fig. 68d); they are discontinuous transversely and longitudinally.

In the abdomen each of the eight segments has beneath the ectoblast (*Ect.*, Fig. 69) a layer of somatic mesoblast (*So. Mes.*) and one of mesentoblast (*Mes. E.*); and at the base of each limb bud (*Ab. 5.B*) there is a cœlom (*Coel.*) formed, as in the thorax, by secondary separation of the layers; right and left sacs of the two sides are separated by the ventral sulcus, but on each side of this groove the mesoblast sacs are longitudinally connected. The first segment, overlooked by so many observers, also has two layers of mesoblast (*Ab. 1*, Fig. 70), which is separated from that of the hindmost thoracal segment (*L. 4*); it has also its own pair of nerve ganglia (*Ab. G. 1*, Figs. 63, 66). Within the caudal lobe (*Caud.*, Fig. 69, Pl. VI) the mesentoblast is still for the most part one-layered.

At this stage appears distinctly, and for the first time, a portion of the definitive entoblast. Its cells lie in the abdominal region between the mesoblast and the vitellocytes, are at first smaller than the vitellocytes, but soon increase in size and develop ramifying processes so as to resemble minature vitellocytes. They are to be found from the first abdominal segment (*Ent.*, Fig. 70) posterior to the caudal lobe (*Ent.*, Fig. 69), and would appear to arise disconnectedly from the mesentoblast in its whole extent. At present the entoblast cells occur sparingly and in small groups. Where they are present three layers can be distinguished between the ectoblast and the vitellocytes: somatic mesoblast (*So. Mes.*, Fig. 70), splanchnic mesoblast (*Sp. Mes.*) and entoblast (*Ent.*). From the account of this and other stages it will be seen that the vitellocytes take no part in producing the entoblast.

Still another process is commencing, the production of blood cells. The extraembryonic blastoderm (Fig. 68d) consists of only one layer, ectoblast, for mesoblast is formed only within the embryonic body, and not until later stages does it grow outward from this body. These extraembryonic cells remained quiescent during the gastrulation period, later proliferated some of the vitellocytes, and now are giving rise to blood cells. Such cells are marked *Bl* in Figs. 68d and 68e, scattered groups or islands of cells produced by multiplica-

tion and enlargement of extraembryonic blastodermic cells in a region where there is no mesoblast. Fig. 67c shows the details of this process on higher magnification, the border of the embryonic body being at the point marked *G. B;* while Fig. 68g shows another group of them *in statu nascendi* just lateral from the head lobe. Their formation is very characteristic and not to be confounded with that of any other cells: at certain points the extraembryonic cells multiply, the nucleus of each enlarges and the cytoplasm still more rapidly, then from the large and dense nucleus strings of chromatin substance pass into the cell body until the latter contains a heavy chromidial net (Figs. 67c, 68g). Thus, the cells come to assume the appearance of those blood cells later found within the heart cavity. These cells are unquestionably ectoblastic, for they arise in regions where there is no mesoblast, and are at the start on the surface of the blastoderm. On the other hand, there are no indications whatsoever of blood cell formation from the mesoblast at this or later stages. As they enlarge they sink below the blastoderm, and the stage following this one will show how they move into the embryonic body by migration. The point of origin of the blood is, accordingly, that extraembryonic area indicated in Figs. 63-66 by stippling.

8. *Stages Immediately Preceding Reversion.*

Here may be considered two slightly different stages, one of about 97 hours (Figs. 71-74, Pl. VI), the other of about 108 hours (Figs. 78, 79, Pl. VII).

The rostral appendages have fused to compose the rostrum (*Ros.,* Figs. 71, 72, Pl. VI; 79, Pl. VII), which is broad with its free end directed anteriorly. Fig. 80b (*Ros.*) shows it on median section and Fig. 75b cut a little to one side of the midline, these figures elucidate also the extent of its rostral mesoblast (*R. Mes.*) in antero-posterior direction; and Figs. 76a (anterior to the rostrum) and 76b (in the plane of the stomodaeum, *Sto.*) in transverse direction. The rostral mesoblast sac (*R. Mes.,* Fig. 80b) is much more extensive than the cheliceral (*Chel. Mes.*). The unpaired rostrum has been produced by the fusion of the paired rostral tubercles of earlier stages.

The stomodaeum (*Sto.,* Figs. 71, 72, Pl. VI; 79, Pl. VII) has

become a hemispherical cup, seen on longitudinal section in Figs. 75b and 80b, and on transverse section in Fig. 76b; it is immediately lined by rostral mesoblast. Its bordering lip is circular, an ectoblastic ring into which projects rostral mesoblast (Fig. 76b).

The cerebral ridges have become more complicated, and on surface views (*Ce. R.*, Figs. 71, 72, Pl. VI; 79, Pl. VII) each is seen to have grown from the stomodaeal region postero-lateral to the antero-lateral vesicle (*A. L. V.*). Posteriorly (toward the stomodaeal side) each ridge is bounded by the fovea (*Fov.*, Figs. 72, 73, Pl. VI; 79, Pl. VII). The fovea has become a deep groove, deepening first mesially, and successive stages of its insinking are shown in Figs. 75b and 80d, Pl. VII (*Fov*). The cerebral ridge is the commencement of the cerebral ganglion, and the fovea, which is its posterior bordering groove, the ventricle of this ganglion. At this stage the cerebral ganglia are therefore invaginating and sinking below the surface.

Lateral and somewhat posterior to each of the preceding ganglionic anlages lies a still deeper and more complicated pit, the antero-lateral vesicle (*A. L. V.*, Figs. 71, 72, Pl. VI; 79, Pl. VII). The cavity of this vesicle is mesially continuous with the fovea (*Fov.*). Where this pit is deepest (*A. L. V.*, Fig. 80c, Pl. VII) its ectoblastic wall (*Ect.*) is greatly thickened. When the antero-lateral vesicle is looked at from the surface in a favorable position (as in Fig. 71, Pl. VI, *A. L. V.*), and before it has joined with the postero-lateral vesicle (*P. L. V.*), it may be described as bounded laterally by a semicircular ridge and mesially by an elevated prominence (*Pr.*). The prominences of both right and left vesicles are lettered *Pr.* in the transverse section represented in Fig. 76a, cut anterior to the rostrum; each prominence is a greatly heightened ridge, with folded outer surface, lying between the fovea and the pit (*A. L. V.*) of the antero-lateral vesicle. This relation is somewhat difficult to describe, but may be understood by comparing the surface view of Fig. 71, Pl. VI, with the section, Fig. 76a, Pl. VII. These projecting knobs of the median walls of the antero-lateral vesicles might suggest, from the examination of surface views alone, that they are additional cephalic appendages; but that they cannot be, for each is simply an ectoblastic elevation that has no special coelomic sac.

The postero-lateral vesicles are exhibited in Fig. 71, Pl. VI (*P. L. V.*) at the earlier stage when they are still separate from the antero-lateral vesicles (*A. L. V.*); a transverse section of them in this stage is shown in Fig. 76b, Pl. VII. Later their anterior margins and the posterior margins of the antero-lateral vesicles grow to meet each other, as shown in Figs. 79 and 80c; Fig. 80a indicates the method of closure of each postero-lateral vesicle by overgrowth of its margin. The antero-lateral and postero-lateral vesicles are the beginnings of the optic ganglia.

Another change is the gradual widening of the ventral sulcus (*Sul. v.,* Figs. 71-74, Pl. VI; 79, Pl. VII), illustrated best, perhaps, by comparison of Figs. 74 and 78. This sulcus has become somewhat diamond-shaped, widest at the juncture of thorax and abdomen, narrowing cephalad as well as caudad. It extends from the stomodaeum to about the caudal lobe, and marks the region where there is no mesoblast. By its widening it occasions a still greater reduction of the extraembryonic area (that part in Figs. 71-73 of which the nuclei are indicated by stippling); and it is the clearest anticipation of the reversion process soon to follow.

The chelicera (*Chel.,* Fig. 72, Pl. VI) and their ganglia (*Chel. G.*) are still poststomial; subsequently each cheliceron acquires a slight maxillary process on its mesial border (Fig. 79, Pl. VII). The maxillary plates of the pedipalps are well marked (Figs. 71, 72, Pl. VI; 79, Pl. VII). The other thoracal appendages (*L.* 1-*L.*4, Figs. 71-74, 78, 79) are becoming longer and more bent, with four or five joints apiece; in the stage of Figs. 78 and 79 those of opposite sides meet ventrally.

The four pairs of abdominal appendages (*Ab. 2b-Ab. 5b,* Figs. 71, 73, 74, Pl. VI; 78, Pl. VII) have grown larger and the two more posterior pairs (prospective spinnerets) are the largest; all are somewhat blunt and rectangular in form; the most posterior pair are shown on section in Fig. 77. The caudal lobe (*Caud.,* Figs. 71-73, Pl. VI; 78, Pl. VII) is short, apically rounded and slightly projecting above the extraembryonic area (Fig. 80e). While the seventh and eighth abdominal segments are at first still distinguishable (*Ab.* 7, *Ab.* 8, Fig. 71, Pl. VI), they later fuse with the caudal lobe (Fig. 78, Pl. VII).

The mesoblast of the cephalic region shows the same general disposition as in the preceding stage (rostral mesoblast, *R. Mes.*, and cheliceral, *Chel. Mes.*, Figs. 75b-76c, 80a-d). The cheliceral mesoblast has grown laterad to some degree and also to some extent beneath the cheliceral ganglia (Fig. 76c), but is still separated from the stomodaeum. The thoracal mesoblastic sacs have grown little save that, owing to the widening of the ventral sulcus, those of the right side have separated further from those of the left, and that those of the same side have come to meet each other longitudinally. Each abdominal appendage has a mesoblast sac with coelom (*Ab. 5.b*, Fig. 77) and so does the caudal lobe (*Caud.*, Figs. 75a, 80e).

The entoblast in the stage of 97 hours has increased and is arranged in scattered groups of cells in the region of the abdominal appendages (*Ent.*, Fig. 77) as well as in the caudal lobe (*Ent.*, Fig. 75a); its disposition indicates continuing formation from the mesentoblast. In the stage of 108 hours it forms a nearly continuous sheet beneath the caudal lobe and the segments immediately anterior to this, seen best on median section (*Ent.*, Fig. 80e); its nuclei are smaller and flatter than those of the splanchnic mesoblast (*Sp. Mes.*).

The extraembryonic blastoderm is continuing the process of proliferating blood cells, and as these cells separate from the blastoderm they migrate upon the yolk to get into the embryonic body. Figs. 75a and 75b show some blood cells (*Bl.*) still extraembryonic, and other larger ones that have moved beneath the caudal lobe. Fig. 80a exhibits a group of them developing just anterior to the head lobe, and Figs. 76a and 80c just lateral to it. Those that have reached the embryonic body lie for the most part between it and the yolk (or vitellocytes), but occasionally some occur within the mesoblast or even the coelom. There is no indication that any of the blood cells are mesoblastic or embryonic in orgin; on the contrary, the centers of formation lie exclusively in the extraembryonic blastoderm, and the latter has now ceased to produce vitellocytes and is forming blood cells only.

The nerve ganglia of the stage of 97 hours are little different from those of the preceding stage, but at 108 hours (Figs. 78, 79) they are hardly distinguishable on surface views. The cheliceral ganglia

are to be seen in Figs. 76c, 80a, 80b, *Chel. G.*, and lie behind the mouth. The pair of the first abdominal segment are transversely wider than those of the other abdominal segments (Figs. 73, 74, Pl. VI; 78, Pl. VII).

9. *The Stage of Reversion.*

Details of this stage are illustrated on Pl. VIII, and the upper row of figures (81-86) represent the external conditions that may be described first.

On comparing Figs. 81-86 of this plate with Figs. 78, 79, Pl. VII, and Figs. 71-74, Pl. VI, it will be seen that reversion consists to great extent in a movement of the caudal lobe to a ventral position almost in line with the fourth pair of legs, together with a shortening of the abdomen. An earlier stage of the process is exhibited in Fig. 81, and a later in Fig. 82, while the amount of the movement may be appreciated by comparing Fig. 84 with Fig. 73. Several other changes are concomitant, to wit: (1) All the abdominal appendages come to lie in approximately the same transverse line with the caudal lobe (Fig. 82); (2) the ventral sulcus (*Sul. v.,* Figs. 81, 82, 84) is much shortened and widened so as to be roughly triangular in outline with the base of the triangle resting against the abdomen; (3) the bases of the thoracal limbs are pushed much further dorsad; (4) the extra-embryonic region has become obliterated save in the dorso-median line (*H.,* Figs. 84-86).

The mechanical causes of reversion will be discussed under the heading, "Summary of Observations."

Previous to reversion the chelicera lay postoral, but now they are anterior even to the rostrum (Figs. 82, 83), as are their ganglia (*Chel. G.*, Figs. 83, 85). The relations of these ganglia to the stomodaeum is shown on longitudinal sections in Figs. 89, 90, 91a, and on transverse sections in Figs. 87a, b; they embrace the stomodaeal tube laterally, are continued anteriorly (dorsally) to it, and are sinking below the ectoblast.

Other notable changes have been effected in the cephalic region. The mouth opening is reduced to a slit (*Sto.,* Fig. 82); the stomodaeum has grown inwards still deeper, it is shown in its full extent in Fig. 90, and its blind inner end is somewhat dilated. The rostrum is to be

seen on longitudinal section in Figs. 89-91a and on surface views in Figs. 82, 83, 85; it has maintained its former position while the chelicera have pushed forward to its level. The cerebral ridges of the earlier stages have sunk beneath the surface to constitute the cerebral ganglia (*Ce. G.*, Figs. 83, 85, 87a, b, 89-91a); they lie further dorso-posterior than the other parts of the brain, and each of them is curved, as best shown in Figs. 85 and 91a. Between them and the rostral surface lie the optic ganglia (*Opt. G.*, Figs. 83, 85, 89, 90) that have been formed by the union of the antero-lateral and postero-lateral vesicles (*A. L. V.*, *P. L. V.*, Fig. 87b). It is somewhat difficult to be sure of the precise relations of these cephalic ganglia on account of the folding and invagination they have undergone, and on the surface views represented in Figs. 83 and 85 they are below the surface, and consequently somewhat obscured. But Fig. 89 indicates their relations on a longitudinal section, and Figs. 87a (in the plane of the stomodaeum) and 87b (anterior to this plane) on two oblique transverse sections of one embryo; the latter two figures show that the cheliceral ganglia (*Chel. G.*) are nearest the midline and embrace the stomodaeum anteriorly and laterally, that the cerebral ganglia (*Ce. G.*) adjoin these dorso-laterally, and that the optic ganglia (*A. L. V.*, *P. L. V.*) touch them ventro-laterally. All these ganglia, as those of the thorax, are developing neuropile (shown in the drawings by stippling).

At this stage appear the antero-median eyes, as ectoblastic infoldings above the rostrum (*M. E.*, Figs. 89, 91a).

In the thoracal region the legs exhibit about five joints apiece (Figs. 81-86), and the maxillary process of the pedipalps is well developed (*Ped. M.*, Fig. 83). The dorsal surface view, Fig. 85, shows how the bases of these extremities have moved much further dorsad, and how by an ectoblastic dorsad growth the extraembryonic region has become reduced to the narrow band of the heart (*H*).

The abdominal region is shown on surface views in Figs. 81-86. Boundary lines between its component segments are seen as divisions in its lateral areas (Figs. 81, 84, 86) demarcating the anterior five segments. Of the four pairs of abdominal appendages (Figs. 81, 82, 84, 86) the third and fourth pairs (*Ab. 4b*, *Ab. 5b*) are much the

largest, and these will form the spinnerets, but have not yet produced glandular ingrowths. The second pair of appendages are the smallest and are connected on each side by an oblique ridge with the first pair (Fig. 86). The first pair of abdominal appendages, those of the second segment, are beginning to invaginate as the lung books; it is interesting to note that primary lamellae arise simultaneously with the deepening of the lung sac, shown on surface view in Fig. 86, and on sections in Figs. 91c, 92, 93, *Pul. C.* denoting the lung cavity and *Pul. L.* the primary lamellae. Fig. 93 is a section through the fourth leg (*L.* 4), the first abdominal ganglion (*Ab.* 1 *G.*), the lung book and the third and fourth abdominal appendages (*Ab. 3b, Ab. 4b*); and Fig. 91c through the lung book and a portion of the third abdominal segment (*Ab.* 3). Figs. 89 and 91a also exhibit the early lung books. Each lung book has three lamellae composed of ectoblast. The tail lobe at the earlier part of the reversion (*Caud.,* Fig. 81) is still somewhat posterior to the appendages, but subsequently (Figs. 82, 84, 86) it moves forward to about their level. Its shape is seen best in Figs. 84 and 85, and its terminal apex is elevated above the surface of the embryo (Fig. 91b). This tail lobe represents the fusion of the caudal lobe proper (telson) with the three posterior abdominal segments. There is still no proctodaeal invagination.

The nerve ganglia of the thorax (Figs. 89, 91a) are contiguous, a composite of those of the pedipalps (*Ped. G.*) and the four legs (*L.* 1 *G.-L.* 4 *G.*). The first abdominal ganglion (*Ab.* 1 *G.*, Fig. 89) seems to be fused with the other abdominal ganglia forming a compound ganglion united with the most posterior thoracal. All these ganglia are still connected with the superficial ectoblast.

All cephalic and thoracal mesoblast sacs are shown in Fig. 91a. The rostral sacs are the largest in the embryo (*R. Coel.,* Figs. 87a, b, 89-91a), they bound the cerebral ganglia (*Ce. G.,* Figs. 89, 91a) posteriorly and these and the cheliceral ganglia laterally (Fig. 87a), are continued into the rostrum (*Ros.,* Figs. 89-91a) and along the dorso-anterior aspect of the stomodaeum. These are very voluminous sacs, but much folded by the invagination of the several parts of the brain. They meet in the midline within the rostrum and along the antero-dorsal border of the stomodaeum (Fig. 90), also dorso-posterior

to the cerebral ganglia (Fig. 87a), where a cleft between them marks the cephalic end of the heart. In comparison with them the cheliceral sacs are small, but little larger than those of the thorax; they are seen on median section in Fig. 91a (*Chel. Coel.*) postero-lateral to the stomodaeum (*Sto.*) and they show a cellular thickening of the wall next this; what this thickening may represent I do not know, unless it be a portion of the poison gland. They are continuous with the rostral sacs only along the stomodaeum. Within the abdomen there are separate coelomic cavities for segments one to five inclusive, but with the fusion of the more posterior abdominal segments their mesoblast sacs have fused to compose a pair that extend into the caudal lobe; these may be detected on surface view (*Caud.*, Fig. 82) and more clearly on transverse section (*Coel.,* Fig. 87c).

With the movement of the caudal lobe and its consequent elevation above the abdominal area (*Caud.,* Figs. 84, 86) it has come to include an axial tube of definitive entoblast. Fig. 87c, *M. G.,* shows this mesenteron on transverse section bounded on either side by mesoblast, and Fig. 91b on longitudinal section. The latter figure illustrates how the inner end of this tube is continuous with a layer of entoblast (*Ent.*) next to the yolk (*Vit.*). The commencement of the mesenteron as a tubular structure is within the caudal lobe, and this tube is anteriorly continuous with a single interrupted entoblastic layer situated at the postero-ventral border of the yolk mass. At no other point in the embryo is there definitive entoblast, but at all places save in a portion of the head region the yolk is bordered by mesoblast; in the head (Fig. 87a) the yolk is divided anteriorly by the rostral mesoblast sacs into a right and left moiety, each placed between a (more mesial) rostral sac and the (more lateral) thoracal sacs, and anteriorly each yolk moiety comes in contact with ectoblast (see the right side of Fig. 87b). Entoblast appears to be absent in the dorsal abdominal region.

The reversion of the embryo with the rapid growth of the dorsal margins of thorax and abdomen have produced the heart. This is lettered *H* in Figs. 82, 84-86, and is a dorso-median tube extending from the cerebral ganglia to the base of the caudal lobe. Intersegmental boundaries represent the beginning of its vessels, and these

are to be seen in Figs. 84-86. At its anterior end, which will become the cephalic artery of the adult, it is bounded by the mesial walls of the rostral mesoblast sacs (Fig. 87a). In the embryo at reversion the pairs of ostia are more numerous than in the adult—about seven or eight in number. An earlier and a later stage in the heart development are shown in Figs. 88 and 87d, respectively, both being portions of transverse sections of the dorsal region. In Fig. 88 right and left coelomic sacs (*Coel.*), each with somatic (*So. Mes.*) and splanchnic layer (*Sp. Mes.*) have approximated, and in the midline between them is an archicoelic space, the heart (*H*), bounded ventrally by yolk (*Vit.*). This early heart space contains blood cells (*Bl.*); and the splanchnic mesoblast on each side of it is thickened to make the beginning of the walls of the heart tube. The later condition is shown in Fig. 87d; the heart cavity (*H.*) is now completely enclosed by splanchnic mesoblast, this being the heart wall, while the coelomic space on either side of it is the pericardial cavity. There is no doubt that at this stage the heart cavity is archicoelic, its wall of splanchnic mesoblast, and the pericardial cavity coelomic in origin. Within the heart lie two kinds of blood cells; smaller cells, the origin of which I have not traced, though there seems to be no evidence of mesoblastic origin, and the large cells with chromidial nets whose history we have learned. Most of these larger cells are now, as before, archicoelic in position, placed between the yolk and the splanchnic mesoblast or between the ectoblast and the somatic mesoblast (Fig. 88). But occasionally they are found within the coelom, as is the case with the most right-hand one of Fig. 87d; this is not surprising, for the mesoblast is discontinuous at many points, and at any one of them a blood cell could pass from the archicoel into the coelom. These blood cells are for the most part dorso-median, within the heart, but many lie right and left of it (Figs. 88, 87d), at the anterior end of the heart there is a crowded mass of them (Fig. 87a) and others also in more lateral positions (Figs. 87a, b, 89-91a) between ectoblast and yolk. But anterior to the caudal lobe there are none in ventral position and relatively few far lateral from the dorso-median line, hence the reversion process has translocated most of them dorsad. The posterior end of the heart is drawn in Fig. 91b, where some of the blood

cells lie between the entoblast and the mesoblast. With the disappearance of the extraembryonic area formation of blood cells seems to end, or at least I found no new centers of proliferation, probably therefore new blood cells are from now on produced by division of the old; perhaps the small cells within the heart have been formed in this way.

10. *The Germ Cells.*

I have given much time in the attempt to trace the origin of the germ cells, but have reached only inconclusive results. In my paper on the fertilization (1907) I described an extranuclear mass near one of the nuclei of the four-cell stage, and suggested that such a body might represent either abnormally placed chromosomes or else a normal chromatin exclusion. "In no other cells of the two-cell, four-cell or eight-cell stage, either in the anaphase or the rest condition, were bodies like these found, so that it is fair to conclude that the two eggs first mentioned were abnormal." Now, I find extruded chromatin masses in most of the cells invaginating at the anterior cumulus, and some of these cells appear to originate within the cytoplasm structures somewhat similar to nuclei, a phenomenon that I propose to treat specially at another time; but similar bodies occur in what appear to be germ cells, so that their presence is not a sign of somatic differentiation.

No evidence of germ cell segregation could be found until the later portion of the gastrulation process, and then in the region of the blastopore of the anterior cumulus. In Fig. 28, Pl. II, is exhibited a cell with a nucleus much larger than those of any other cells (*G. C.* ?); it borders on the gastrocoel, and may be the first definitive germ cell, but the only reason for so supposing is the great size of its nucleus. In the same situation is found a little later a group of eight cells immediately lining the gastrocoel (*G. C.* ?, Figs. 37b, 38b, Pl. III); these are unbranched, thus differing from the early vitellocytes, they possess relatively clear nuclei and are much larger than the early mesentoblastic cells (*Mes. E.*, Fig. 37a). With the obliteration of the gastrocoel and the flattening of the anterior cumulus these cells become indistinguishable, so that they either become branched like vitellocytes or else by division become as small as the mesento-

blast cells. At the stage of the early abdominal segments are found in the abdominal midline separated patches of small cells (*G. C.* ?, Figs. 54, 56b, 61, Pl. V); these may be definitive entoblast, but differ from the entoblast of later stages in being branched and from the vitellocytes in their much smaller size, therefore they may be germ cells. In later stages I could not distinguish germ cells from entoblast, and the genital organs arise considerably later than the stage of reversion.

II. Summary of Observations With Comparison of the Literature.

11. *Cleavage Up to Gastrulation.*

The egg before segmentation shows cytoplasm around the central pronuclei, and a delicate network, placed between the yolk globules, connecting this central cytoplasmic mass with a fine peripheral layer. The yolk consists of an outer layer of radial pyramids and an inner layer of large granules not so disposed; I have not specially studied the segmentation of the yolk during cleavage because the fixation employed coagulated the yolk in the earlier stages. Cleavage consists in repeated nuclear divisions, the nuclei as they become more numerous move nearer and nearer to the surface of the egg, whereby the central cytoplasmic mass divides into as many portions as there are nuclei and the intravitellar cytoplasmic network shortens until all nuclei and all cytoplasm become placed on the surface. During the earlier cleavage a central fluid mass forms in the egg. Inequality in rate of nuclear division commences at the 32-cell stage. At the stage of 140 cells all the nuclei have become superficial, equally numerous at all points on the surface, thus forming the early blastoderm. No nuclei remain in the yolk and no polarity of the egg can be distinguished up to this stage. On the blastoderm the ventral embryonic area (germ disc) becomes established by more rapid multiplication of nuclei in that region and by migration of other nuclei toward that pole; then for the first time appear distinct cell membranes, and these form gradually as the separate cytoplasmic masses come in apposition. The superficial cytoplasmic layer is not divided into cell areas before this period.

In much of the preceding work more attention had been given to

the changes in the yolk pyramids than to the cytoplasm and nuclei. Claparède (1862) found the blastoderm to be at first a single layer of cells; and Salensky (1871, *Theridium*) and Ludwig (1876, *Philodromus*) described the yolk pyramids and movement of the cleavage nuclei to the surface. Then Balbiani (1873, *Agelena, Tegenaria, Epeira*) studied the cleavage and concluded that the cleavage nuclei move into particular preformed cell territories of the superficial cytoplasmic layer (couche germinative); this particular conclusion of Balbiani's careful memoir has not been substantiated by subsequent students. Balfour (1880) believed each yolk segment to be a cell and the cytoplasm to consist of an envelope for each nucleus and a reticulum around the yolk granules. Sabatier (1881) has added nothing of importance to our knowledge. Locy (1886, *Agelena*) showed that the supposed cell territories of Balbiani are the result of the pressure of yolk columns upon the superficial blastema, and that these do not come to coincide with the later blastoderm cells; he found also that the cleavage nuclei reach one pole of the egg first, that called by him the "animal-pole," and that all the nuclei are derivatives of a single original one. He is the only observer to note a polarity of the egg before cleavage: "One hemisphere is characterized by small yolk corpuscles packed closely together, though not joined in masses, and the other by agglomerations of larger yolk corpuscles."

Morin (1887) described a central cytoplasmic mass with fine strands radiating from it, showed that at the eight-cell stage the yolk divides into eight equal masses placed around a cleavage cavity; then these divide further following division of their nuclei, forming rosettes, and ultimately all the cells reach the surface of the egg. Schimkewitsch (1887) corroborated in some points Ludwig and Locy, found that the number of yolk pyramids at the end of the cleavage varies with the species; there is no independent superficial layer of cytoplasm, but the protoplasm occupies the center of the egg; later (1898) he showed, correcting his conclusions of 1887, that all the cleavage nuclei reach the surface to form the blastoderm, none remaining within the yolk. Kishinouye (1890) calls the central cytoplasm the "centroplasm," and the peripheral, the "periplasm,"

he finds that the yolk columns are as numerous as the nuclei, and that at a stage of about 30 cells all the cells reach the surface to constitute the blastoderm, their cytoplasm masses then fusing with the periplasm.

12. *Gastrulation and Formation of the Germ Layers.*

There first forms a thickening of the germ disc at a point slightly posterior to its center, this is the anterior cumulus, and it becomes only slightly elevated above the surface of the egg. It is at first circular, with a shallow circular gastrocoel, and rapid cell proliferation takes place from it. This anterior cumulus proliferates first vitellocytes, branched highly vacuolated cells that ingest yolk rapidly and some of which sink into the yolk, while most wander along its surface just beneath the germ disc; and second, mesoblast and mesentoblast cells that scatter upon the surface of the yolk. A second or posterior cumulus arises posterior to the former and somewhat later in time, usually at the posterior edge of the germ disc; it is more elevated and prominent, and its thin anterior border marks the future thoraco-abdominal boundary; it has no gastrocoel and proliferates only vitellocytes. The two cumuli become later connected by movement of vitellocytes between them. Still other vitellocytes arise at the anterior and lateral margins of the germ disc. The gastrulation process is accordingly threefold: (1) at an anterior and (2) a posterior cumulus, and (3) at the anterior and lateral margins of the germ disc, from all of which points arise vitellocytes, but only from the first does mesoblast and mesentoblast originate. The layer between ectoblast and vitellocytes seems to have exclusive origin from a group of about eight cells placed at the anterior cumulus, the descendants of these eight cells moving in all directions beneath the germ disc; in the protozonite stage this layer is only one cell deep, and within the cephalothorax it is true mesoblast, but in the abdominal area it is mesentoblast. The mesoblast of the cephalothorax splits into a somatic and a splanchnic layer. The mesentoblast of the abdomen extends along its whole length, and is not limited to the caudal lobe; it splits first into an outer layer of somatic mesoblast and an inner layer of mesentoblast, then the inner layer separates into splanchnic

mesoblast and definite entoblast. It is not until the stage of the early abdominal limb buds that the entoblast distinctly segregates from the mesoblast in the abdominal region, and it is not a continuous layer; not until the stage of reversion does the entoblast form a tubular mid-gut, then only within the caudal lobe and in consequence of the elevation of this lobe above the embryo, while even at the stage of reversion the entoblast is still a discontinuous layer in the abdomen anterior to the caudal lobe. The definite entoblast arises only abdominal, none of it originates with the cephalothorax, for the whole gut (stomodaeum) of the cephalothorax is ectoblastic; it segregates relatively late and even at the time of reversion lines the yolk only posteriorly and ventrally. The cephalothorax possesses only ectoblast and mesoblast. The vitellocytes do not form entoblast, and though my study does not include stages late enough for me to decide this point, the evidence is that the vitellocytes take no part in the formation of the intestine, but play much the same rôle as the periplast (periblast) cells of vertebrates. After the gastrulation new vitellocytes are formed from the extraembryonic blastoderm.

Claparède (1862) described a central "cumulus primitif," which is at first a rounded eminence, then elongates to pyriform shape, extending back to the "pôle anal" and proliferating cells along its whole length; at the stage of the early segments he identified it with an eminence placed dorsally between head and tail lobes. Then Salensky (1871, *Theridium*) found a funnel-like invagination developing in the middle of the germ disc, the opening of which finally closes, and the cells proliferated from it wander beneath the whole blastoderm; before this invagination closes there arises behind it a transitory mound of blastoderm cells. Balbiani (1873) corroborated Claparède in the appearance of a primitive cumulus near the center of the germ disc on the more flattened surface of the egg; at that point the blastoderm is more than one cell layer deep, and it is the center of that thickened portion that gradually elevates itself; near it arises by cell proliferation a wider area, the "tache postérieure," distinct from the cumulus; the "tache postérieure" represents the cephalic lobe, while the cumulus takes a dorsal position (dorso-caudad from the cephalic lobe). Balfour

(1880, *Agelena*) was the first to employ actual sections in the study of these relations; he found a cumulus appearing near the edge of the germ disc ("ventral plate"), near the center a larger "white area," and determined that a line connecting the two marks the future long axis; he is inclined to identify the white area with the future procephalic lobe, and the cumulus with the caudal lobe. Cell invagination takes place at least in the region of the white area, and the cumulus is "the point where the first traces of the future mesoblast become visible." The thickening of these areas is due mainly to cell multiplication, but in part also to addition of cells from the yolk. The ventral plate then divides into epiblast and mesoblast; the splanchnic mesoblast becomes formed by yolk cells adding themselves to the ventral plate, and so probably arises the intestine (that appears much later). According to Balfour, accordingly, both splanchnic mesoblast and entoblast are formed from yolk cells adding themselves to the embryo. Locy (1886) found a depression succeeded by a primitive cumulus "at one end of the flattened surface of the egg * * * a second thickening, which I shall call the caudal thickening, now makes its appearance on the flattened surface of the egg, at a distance of about 80° from the cumulus." It increases rapidly in size, spreading out most in the direction of the cumulus, and ultimately becomes shield-shaped. In the region between these two structures the ventral plate is gradually formed by a blastodermic thickening, which is not at first continuous with the two terminal thickenings. Cell proliferation occurs in the region of both eminences. In the stage of the protozonites the embryonic area consists of ectoderm and mesoderm, but Locy has not presented detailed observations on the segregation of the two mesoblast layers and first appearance of the entoblast. Lendl (1886) observed on the blastoderm a "Primitivhügel" that comes to take a caudal position, and a "weisser Fleck"; the yolk cells compose the entoblast. Morin (1887) found that from the center of the embryonic thickening cells separate themselves, some to enter into the yolk as entoblast cells and others to remain upon the yolk as mesoblast. The cumulus of the authors is present in *Pholcus* and *Drassus*, but absent in *Theridion,* and arises after the germ layers are well formed; it consists of a mass of mesoblast cells, later it sepa-

rates from the mesoblast to occupy a dorsal position and give rise to blood cells. Bruce (1887) described the primitive cumulus as well formed before the blastoderm is complete. "It is very probably formed both by the division of cells which have reached the surface and by the addition to these of yolk cells"; from it comes the mesoblast. Kishinouye (1890) stated that near the center of the blastoderm there arises a "primary thickening" that is to be compared with a blastopore, and a little later a "secondary thickening"; the former in widening pushes the latter to the margin of the germ disc (but whether the latter lies anteriorly or posteriorly is not stated). The germ layers form from both thickenings, but more specially from the primary. "Cells * * * proliferate into the yolk and become scattered without any definite arrangement through the entire yolk. These are the entoderm cells." Then Schimkewitsch (1887, 1898, 1906) has given the most detailed study of all. All the cleavage cells reach the surface to constitute the blastoderm, then some of them (yolk cells or vitellophags) sink secondarily into the yolk. A cumulus primitivus arises at the middle of the blastoderm, and then a "zweiter Fleck" before (*sic!*) this; these become later connected. The cumulus is the point of formation of the mesentoblast and also of additional yolk cells, along the line of a lengthened blastopore. In the anterior region of the embryo the mesoblast and entoblast become early distinct from each other, while at the posterior end (caudal lobe) embryonic mesentoblast continues to proliferate mesoblast and entoblast. From these and from the vitellocytes Schimkewitsch distinguishes mesoblastic phagocytes, cells that arise wherever mesoblast cells touch the yolk; they are smaller than the vitellocytes, but, like these, ingest yolk. The dorsal cumulus of Claparède and Morin is held by Schimkewitsch to be a true tubular dorsal organ and not a part of the blastopore. The entoblast consists of the posterior anlage, from which arise the cloacal sac, the malpighian vessels and the intestinal epithelia; and of scattered cells on the yolk surface that form the epithelia of the liver sacs.

It will be seen, then, that there is much confusion with regard to the formation of the germ layers. The consensus of opinion, opposed only by Balfour and Bruce, is that the yolk cells sink from

the blastoderm into the yolk secondarily, and with this view my conclusions agree. Then, I agree with Schimkewitsch, in contradiction to the other writers, that the yolk cells do not originate the entoblast or take part in the formation of the embryo; but my results differ from those of Schimkewitsch in finding that no entoblast arises within the cephalothorax, and in finding no good distinction between vitellocytes and phagocytes—perhaps because I have not studied the later stages when the phagocytes are most conspicuous. There is also confusion with regard to the two "cumuli." What I have called the "anterior cumulus" evidently corresponds to the "cumulus" of Claparède (before segmentation of the embryo), the anterior invagination of Salensky, the "white area" of Balfour, the "weisser Fleck" of Lendl, and the "cumulus" of Schimkewitsch, this having a more central position and forming earlier; while my "posterior cumulus" would correspond with the "posterior mound" of Salensky, the "cumulus" of Balfour, the "Primitivhügel" of Lendl, the "secondary thickening" of Kishinouye, and the "weisser Fleck" of Schimkewitsch. In this comparison I disagree with Schimkewitsch's conclusion that his "weisser Fleck" arises anterior to the anterior cumulus, while I agree with him that the so-called "cumulus" of Morin is, as the one described by Claparède after the appearance of the segments, incomparable with the gastrulation cumuli. Balbiani and Locy also observed two cumuli, but since they failed to indicate the boundaries of the germ disc it is difficult to understand their accounts. My conclusion is peculiar that only the anterior cumulus forms mesentoblast.

13. *Segmentation and Appendages of the Cephalothorax.*

The mesoblast at the termination of gastrulation is a continuous single layer; its later segmentation occasions the appearance of the protozonites (early somites). The germ disc changes from a circular to an ovoid outline, then its broader end marks the region of the head, and its narrower end (caudal lobe) marks the position of the earlier posterior cumulus. The earliest stage seen of segmentation was one with four protozonites, which seem to appear almost if not quite synchronously; these are the segments of either (1) the four

ambulatory appendages, or (2) the pedipalps and three anterior ambulatory appendages; the latter alternative is probably the correct one, for the stage with five segments (pedipalpal and four ambulatory) shows the most posterior more distinct than the most anterior. The cheliceral segment does not abstrict from the posterior margin of the head lobe until the other segments exhibit beginnings of appendages. The appendages are produced by lateral movement of the mesoblast away from the midline, this occasioning also the clear median line or ventral sulcus (region from which the mesoblast has withdrawn); with this division of the mesoblast is formed a pair of coelomic sacs for each of the segments mentioned. The stomodaeum arises as an ectoblastic invagination of the head lobe anterior to its middle point; its external lips are mere ectoblastic thickenings, distinct at first from the rostrum; it is at first anterior to the chelicera and their ganglia, but the latter move anterior to it at the time of reversion. The head lobe contains two pairs of coelomic sacs distinct from the start: the more posterior and smaller cheliceral sacs, and the more anterior and much more voluminous rostral sacs. The head region possesses, accordingly, two segments, demarcable not externally, but by mesoblast sacs: the more anterior and larger of these segments is the rostral, and as its appendages are to be considered the two small rostral tubercles, which arise later than the chelicera as a pair of small tubercles just anterior to the stomodaeum, and which now fuse to make the prestomial rostrum; the rostral coelomic sacs extend into their bases. The rostrum, accordingly, represents a pair of true preoral appendages, of which the rostral sacs constitute the coelom and the cerebral ganglia the neuromeres. There is no evidence of other preoral appendages. The cephalothorax consists of seven segments. The mesoblast sacs of the cephalothorax are at first latero-ventral in position and (except those of the rostrum) with coelomic spaces only in the region of the appendages; gradually they extend dorsad, but it is mainly the reversion of the embryo that brings those of opposite sides in proximity on the dorsal aspect of the embryo. Of the appendages both chelicera and pedipalps develop maxillary processes, but those of only the pedipalps persist into the adult.

Claparède (1862) saw no segmented stage earlier than that of six cephalothoracic segments, and described these as appearing simultaneously; the rostrum comes from an unpaired anlage. Balbiani (1873) found the earliest segments to be the pedipalpal and the segments of the first two leg pairs (these nearly synchronously), followed by those of the third and fourth legs, then by the most anterior (cheliceral) which arises from the cephalic lobe. Balfour (1880) described a stage with procephalic and caudal lobes "and about three segments between the two," which segments, he considered, are probably those of the first three leg pairs; the pedipalpal and cheliceral segments are later in origin, and the cheliceral cuts off from the head lobe. The mesoblast of the head lobe (procephalic lobe) is separated from that of the chelicera, and the two coelomic sacs that compose it are connected in the midline around the stomodaeum. "The procephalic lobe represents the preoral lobe of Chaetopod larvae." The chelicera are postoral in position, arising behind the ectoblastic stomodaeum, "and terminate in what appear like rudimentary chelae"—an observation shown by Schimkewitsch to be erroneous. Croneberg (1880) found that the rostrum is formed from a paired anlage, and equals at least one pair of extremities. Locy (1886) observed the earliest segmental stage to consist of "three zonites and the cephalic plate. * * * The addition of new zonites to those already existing goes on rapidly; the two anterior ones (those of the chelicerae and the pedipalpi) are cut off from the posterior end of the cephalic plate. * * * The other zonites are developed from the caudal plate." * * * A "prominent upper lip composed of two lateral elements" later arises just anterior to the stomodaeum. The ventral sulcus is produced by the mesoblast separating medio-ventrally. Lendl (1886) alone claimed to observe a segment arising between the cheliceral and the pedipalpal; this "mandibular" segment is said to develop a pair of "mandibles" that later fuse with the unpaired rostrum. Morin (1887) described the germ disc as becoming triangular, the broader end of which (Vorderlappen) represents the anlage of the cephalothorax; the first segment to appear in the sixth, then the fifth, and so on from behind forward, the cheliceral last; there is an independent pair of coelomic

sacs for the head lobe. Schimkewitsch (1887) described the rostrum ("lèvre supérieure") arising as a pair of tubercles, basally contiguous, just anterior to the mouth; a similar pair of tubercles arising later ("lèvre inférieure") border the mouth posteriorly; the rostrum forms by coalescence of these two pairs. The mesoblast of the cephalic lobe is continuous with that of the chelicera, but later separates from it. All the cephalothoracic segments appear simultaneously except the cheliceral. Jaworowski (1891) described in *Trochosa* as "antennae" a pair of small unjointed prominences immediately anterior to the chelicera, but as seen on surface views only; he did not decide whether the "Oberlippe" (rostrum) is paired in origin. In this and a later paper (1892) he brings evidence to show the cleft, crustacean, nature of arachnid extremities. Kishinouye (1894) found the mesoblast of the head lobe continuous with that of the chelicera, and though a pair of coelomic spaces develop for the chelicera and two pairs later for the head lobe, the wall of all of them remains continuous. Pokrowsky (1899, *Pholcus*) described briefly, and from a surface view only, two pairs of "Kopfhöcker" at a stage when the limbs are jointed; the anterior lie above the antero-lateral vesicle, and the posterior (which he compares with the "antennae" of Jaworowsky) somewhat posterior to them; he figured the rostrum as bilobed. Pappenheim (1903, *Dolomedes*) described the coelomic sacs of the cephalothorax, and found that of the cephalic lobe to be from the start separate from that of the chelicera; the "upper lip" and "lower lip" are differentiations of an unpaired prominence in front of the mouth; Pappenheim opposed the existence of the preoral extremities of Jaworowsky and Pokrowsky. Wallstabe (1908) finds there is a separate head coelom; and he also discovered a special egg tooth near the base of each pedipalp.

The special points in which I have compared the results of other investigators are (1) the order of appearance of the cephalothoracal appendages and segments, (2) the matter of an independent mesoblast in the cephalic lobe, and (3) the matter of appendages on the cephalic lobe. All observers seem to agree that the cheliceral segment arises latest in a postoral position by separating from the head lobe, and that at reversion the chelicera move anterior to the mouth.

My observations would show that the segments of the pedipalps and legs arise almost synchronously in accord with most of the preceding work, while Balbiani held that the segments of the third and fourth legs appear later than the other thoracal segments, and Morin held that they developed in order from behind forward; there seems to be no good evidence for Morin's view, nor yet for Lendl's contention that a segment appears between the cheliceral and the pedipalpal. My results are in agreement with those of Balfour and Pappenheim, and in opposition to all others, that the mesoblast of the head lobe is separated from that of the cheliceral segment before appendages appear. My views uphold also that of Croneberg, that the rostrum has paired origin and is to be considered a pair of true preoral appendages. But, with most other observers, I agree that there are no other true cephalic appendages; the "antennae" of Jaworowsky seem to be simply basal portions of the chelicera, composing what is a temporary maxillary plate; and the "head tubercles" of Pokrowsky evidently correspond to the walls of the anterior and posterior lateral vesicles of the head lobe, which are ganglionic formations. Certain it is that there are not more than one pair of coelomic sacs within the head lobe, anterior to the cheliceral sacs.

If the attempt be made to institute a comparison with the Crustacea, then the chelicera of the arachnids might be considered homologous with the second antennae of the crustacean, for both arise postoral then move in front of the mouth, and this view was expressed by Korschelt and Heider (1892); therefore, the rostral appendages of the arachnid, which are preoral in position, might correspond with the first antennae of the crustacean.

14. *Segmentation and Appendages of the Abdomen.*

The abdomen grows in length rapidly, after the early thoracal segments have appeared, by teloblastic growth, and its segments appear successively from its anterior end backward. The posterior caudal lobe is a true telson. Eight segments are developed anterior to the caudal lobe, but at reversion the three posterior segments fuse with this lobe. Of these segments the second to the fifth inclusive develop limb buds, the first pair becoming the lung books and the

fourth and fifth becoming the spinnerets. Lamellae appear on the posterior surfaces of the appendages of the second segment before these appendages invaginate. At reversion the fourth and fifth pairs of limb buds have not begun to proliferate spinning glands. Each pair of limb buds develops a pair of coelomic sacs, and so does the caudal lobe, but in segments 6-8 such sacs do not become clearly distinguishable; such sacs are in part occasioned by withdrawal of mesoblast from the midline forming the ventral sulcus. At reversion these sacs come to meet in the dorso-median line.

With regard to the number of abdominal segments the authors differ. Thus, anterior to the caudal lobe proper Claparède (1862) found in *Pholcus* eight segments and Schimkewitsch (1887) twelve; Barrois (1878, *Epeira*) found nine; for *Agelena* Balfour (1880) observed "probably" nine, Locy (1886) "at least" ten, and Kishinouye (1890, 1894) eight; Jaworowsky (1891) found twelve in *Trochosa,* and Pappenheim (1903) nine or ten in *Dolomedes.* Twelve is the highest number given for any case and eight the lowest; doubtless the number may differ in different species and be larger in the more generalized, though the individual opinion of what constitutes a segment probably introduces an error into the count. Barrois, Balfour, Morin and Locy overlooked the first segment. Kishinouye (1894) has given one of the most thorough accounts of the coelomic sacs: "The last three abdominal segments (sixth to eighth) gradually degenerate and their coelomic cavities seem to fuse together into one pair. The pair of coelomic cavities thus formed by fusion is pushed into the protuberance of the tail as the process of reversion proceeds." Kishinouye found also that the first abdominal segment disappears; and Bruce (1887) described "posterior to the last thoracic appendages a swelling. It corresponds in position to the operculum of Limulus"; judging from Bruce's figure, this is to be considered the first ganglion. Wallstabe (1908) found in *Agelena* nine abdominal segments anterior to the tail lobe, with a pair of coelomic sacs for each.

Four pairs of abdominal appendages placed on segments two to five inclusive were described by Salensky, Schimkewitsch, Kishinouye, Wallstabe and Pappenheim, four pairs were seen also by Bar-

rois, Balfour, Locy and Morin, but assigned by them to segments one to four inclusive, for these writers overlooked the first segment. But other appendages, mostly of smaller size, have been observed. Thus, Claparède saw six pairs in *Clubiona,* while Korschelt (1892) found in an undetermined species appendages on the first segment as large as those of the four following segments, and in *Agelena* slightly developed appendages on the first segment. Then Jaworowsky (1892, 1895) noted pairs of appendages on segments one to eight (the appendages of the first and sixth to eighth segments being very small. I may add that I have found more than four pairs in a species of *Loxosceles,* on which I intend to report in another paper. The observers rightly conclude that the larger number of appendages denotes a more ancestral condition.

As to the fate of these appendages there is some conflict of opinion. Balfour (1880) stated that at complete reversion the "four rudimentary appendages have disappeared, unless—which seems to me in the highest degree improbable—they remain as the spinning mammillae, two pairs of which are now present"; and Schimkewitsch (1887) also maintained that the abdominal appendages disappear and that the spinnerets are new formations. Salensky (1871) first showed that the appendages of the second segment become the lung books, and this important discovery has been corroborated by Locy, Morin, Kishinouye, Jaworowsky, Simmons and Purcell. Wallstabe corroborates Simmons' results, but without giving details. Bruce held that "probably two abdominal appendages are invaginated to form each lung book," meaning thereby those of the second and third segments; but he was mistaken in this and also in identifying a slight fold of the floor of the pulmonary invagination with a pulmonary lamella. Simmons (1894) and Purcell (1895) correctly described the origin of lung lamellae on the posterior surfaces of the appendages of the second segment, before these appendages have invaginated, but I shall show in another paper that these primary lamellae do not become the lung lamellae of the adult; Jaworowsky (1892) found these appendages to become the opercula of the lung books, and studied (1894) the development of their lamellae at stages later than reversion. The appendages of the third segment were found

by Salensky to compose a vascular sinus, while Balfour, Locy, Morin, Schimkewitsch, Jaworowsky found they simply disappear, and Korschelt suggested they might form the tracheae. The appendages of the fourth and fifth segments become the spinnerets, as found by Salensky and corroborated by Locy, Morin, Kishinouye, Wallstabe and Jaworowsky (1895); the last author has given the most detailed account of them, concluding the appendages of the fourth segment form the anterior spinnerets and the cribellum (or colulus), and those of the fifth segment the median and posterior spinnerets.

15. *Growth Differences of Cephalothorax and Abdomen.*

The cephalothorax and abdomen show certain striking differences in their growth and differentiation. The boundary between the two can be recognized as a thin region of the ectoblast, a little anterior to the posterior cumulus at an early stage before there is any trace of segmentation, the posterior cumulus practically coinciding with the position of the caudal lobe. Thus, nearly the whole extent of the cephalothorax is laid down, thereafter to lengthen but little more, when the abdomen is represented only by its future posterior end. Then with the segmentation of the mesoblast the abdomen commences to increase rapidly in length, pushing around upon the yolk until the caudal lobe finally comes into contact with the head lobe. The abdomen is in most of its extent later in origin than the cephalothorax, and its growth is strictly teloblastic by the formation of successive segments from its anterior end caudad; its rapid increase in length comes suddenly at the close of the gastrulation period. This rapid elongation of the abdomen explains why the caudal lobe is narrower than the head lobe. Segmentation appears first in the cephalothorax, and its five posterior segments are well marked before the first abdominal becomes evident; further, these five cephalothoracic segments seen to appear simultaneously or nearly so and not in sequence from before backward, while the cheliceral segment, the most anterior, is the one to form latest. Therefore the segmentation of the embryo as a whole is not telóblastic, but only that of the abdomen; regular teloblastic growth takes place only between cephalothorax and the caudal lobe. Another and perhaps

more striking difference is that the cephalothorax develops no entoblast and of the alimentary tract possesses simply the ectoblastic stomodaeum—it is composed only of ectoblast and mesoblast; a smaller difference is that the cephalothoracal extremities (with the exception of the rostrum) arise at the lateral margins of their segments while the abdominal extremities are more mesial in position. The abdomen persists until a late stage as the more embryonic portion.

These growth differences have been indicated by previous observers, though not specially insisted upon.

16. *Central Nervous System.*

The nerve ganglia arise later than the somites and are hardly perceptible until the first abdominal segments are formed; they are paired ectoblastic thickenings. There is a separate pair for the chelicera (distinct from the cerebral ganglia), for the pedipalps, for each leg pair, and for each of the seven anterior abdominal segments, or thirteen in all, exclusive of those of the head lobe. The anlages of the central ganglia are the paired cerebral ridges with the groove (fovea) behind each; these are situated at first at the antero-mesial border of each half of the head lobe. On each half of this lobe, but more lateral, develops a little later an invagination with heightened wall, the antero-lateral vesicle, and behind the latter a second smaller pit, the postero-lateral vesicle; ultimately each half of the head lobe comes to possess an obliquely transverse ridge, which forms one common border to the fovea and the lateral vesicles. The fovea and the antero-lateral and postero-lateral vesicles are thus at first separate invaginations, and for that reason might be considered separate neuromeres; but all three are inpushings of the one common ectoblastic thickening, the head lobe, all become subsequently continuous and there is only one mesoblastic somite (the rostral) beneath them; therefore, it seems best to consider the head lobe composed of but one pair of neuromeres. These sink beneath the surface, the cerebral ridges and foveae to form the (most dorsal and posterior) cerebral ganglia, and the antero-lateral and postero-lateral vesicles to form the (more lateral) optic ganglia. At the stage of reversion the

cheliceral ganglia push forward to add themselves to the brain, immediately bounding the stomodaeum dorso-laterally and thus forming the oesophageal commissures. The rostrum does not appear to possess separate ganglia. At the stage of reversion the neuropile is developing, though still small in amount. At this stage also the antero-median eyes arise as ectoblastic invaginations above the rostrum.

Claparède described late stages of the eye development. Salensky (1871) was the first to describe carefully the nervous system; he found the semicircular grooves (foveae) of the cephalic lobes, found the cerebral ganglia to arise by invagination of these lobes, and saw eight pairs of ganglia develop in the cephalothorax from the ectoblast, but none in the abdomen. Balfour (1880) described correctly the origin of the nerve ganglia from the ectoblast, in abdomen and cephalothorax, found the cheliceral ganglia arise "quite independent of the procephalic lobes," and observed that the greater part of the latter "is destined to give rise to the supra-oesophageal ganglia." On the anterior border of each procephalic lobe he distinguished a transverse "semicircular groove." He showed that at reversion of the embryo the cheliceral ganglia became part of the oesophagaeal commissures, and that the abdominal ganglia fuse together. Locy (1886) confirmed Balfour's results, and added to it a full description of the development of the eyes. Morin (1887) saw on the cephalic lobes two semicircular grooves which close, sink below the ectoblast and "join with the brain." Bruce (1887) described folds on the head lobe which he called "amniotic folds"; his figures show clearly that he was describing the lateral vesicles. Schimkewitsch (1887) held the cheliceral ganglia of Balfour to be the basal joint of the chelicera and stated (erroneously) that none of the cephalothoracal ganglia can be seen on surface views. He gave a detailed description of the nervous system; twelve abdominal ganglion pairs develop in *Pholcus;* a separate ganglion pair for the rostrum, and in the abdomen a median ganglionic thickening besides the lateral ones. Schimkewitsch then made an extended comparison of the nervous system with that of other groups. Kishinouye (1890, 1894) found the semicircular grooves of Balfour, called the part of the cephalic lobe

anterior to each the "semicircular lobe" (this equivalent to what I have called the cerebral ridge), and found the semicircular grooves become the chief part of the "brain"; he also found that the "lateral vesicles" compose a part of the "brain," and described the origin of the eyes. Pappenheim (1903) reached conclusions that are in close agreement with my own; from his fovea semicircularis of the head lobe arises the cerebral ganglia, from the vesicula lateralis the optic ganglion, and from the cheliceral ganglia the oesophageal commissures; he observed the abdomen to have independent ganglion pairs for the first six segments, and three or four fused ganglionic pairs behind those—the abdomen thus possessing more neuromeres than somites.

17. *Blood Cells and Heart.*

The blood cells first differentiate when limb buds begin to appear on the abdominal segments, and they develop entirely from the extraembryonic blastoderm. This area is the one lateral and dorsal from the embryonic body which consists of but one layer of cells, ectoblast, and which previous to the proliferation of blood cells had furnished those vitellocytes that are formed after gastrulation. At numerous points, blood islands, of the extraembryonic ectoblast the cells increase in size and the chromatin of their nuclei enters the cytoplasm to compose coarse chromidia; such cells then sink beneath the ectoblast to lie upon the yolk. These blood cells are the largest of all the cells and have exclusive origin from the extraembryonic ectoblast in regions where there is no mesoblast. Subsequently they move along the surface of the yolk into the embryonic region, where they come to lie for the most part between the yolk and the embryonic body, though some of them may penetrate into the mesoblast or even into the coelom. At reversion the extraembryonic area becomes obliterated by overgrowth of the body around the yolk, and then ceases the formation of blood cells from the blastoderm. This reversion narrows the extraembryonic area to a dorso-median space, the cavity of the heart, bounded on either side by the dorsal ends of the somites; these somite walls then overreach and underreach the blastocoelic heart cavity and the wall of the heart tube is a product of splanchnic mesoblast; the coelomic cavity lateral of the heart tube is the begin-

ning of the pericardial cavity. With the formation of the heart most of the large blood cells become placed within its cavity. The heart is formed very rapidly as a continuous dorsal tube extending from the brain region (cephalic artery) caudad to the caudal lobe; the dissepiments (transverse spaces between segments) constitute its earliest vessels or ostia, and there are at reversion seven or eight pairs of these.

Claparède (1862) and Salensky (1871) found the heart to be solid at first, and its core later changing to blood corpuscles. Balfour (1880) reached the same conclusion, and stated the heart to be mesoblastic "formed before the dorsal mesoblast has become differentiated into two layers." The cells which I have described as blood cells he considered derivatives of yolk cells. Locy (1886) was "convinced that it is not, as Balfour states, developed from a solid cord of cells, but from the dorsal limb of the upgrowing mesoderm, and that its dorsal wall is closed first." He maintained that the aorta developed later from the mesenteron, and that the blood cells are derivatives of yolk cells. Morin (1887) found large extraembryonic cells ("links und rechts vom Keimstreifen") between ectoblast and yolk that become blood cells, and he supposed they arise from mesoblast. With the dorsal growth of the mesoblastic somites at reversion these large cells come to lie dorso-median, and the heart wall is formed by the adjacent mesoblast, the heart cavity being archicoelic. Schimkewitsch (1887) held the blood cells are derivatives of "secondary entoblast," which forms from yolk cells that become round, reach the surface of the yolk and get into coelomic cavities; ultimately these become placed within the heart, the development of which is as Morin described. Kishinouye (1890) corroborated Morin's description of the heart formation, and found (1894) that "the number of the slits in the adult heart shows approximately the number of the segments which took part in the formation of the heart"; namely, the second to the fifth abdominal segments inclusive.

The heart cavity is archicoelic, its wall mesoblastic, and the pericardial cavity coelomic, as Morin, Schimkewitsch and Kishinouye have insisted. But all the writers have failed to observe the very characteristic formation of blood cells from the extraembryonic ectoblast.

18. *The Germ Cells.*

Assumed germ cells were (1) an unusually large cell in the vicinity of the anterior cumulus at the late gastrulation, succeeded by (2) a group of some eight large cells immediately bordering the gastrocoel, which resemble at least in position the germ cells of the Scorpion as described by Brauer (1894), and (3) discontinuous median masses of small cells in the abdominal region before reversion. At reversion no germ cells can be recognized with certainty.

None of the observers, Schimkewitsch, Kishinouye, Purcell, Strand, who have described the germ glands and genital ducts distinguished germ cells until after the stage of reversion.

19. *Movement of Parts During Differentiation.*

The movements of the protoplasm during the course of development are very prominent and full of interest from the mechanical side. In the following lines I shall attempt to briefly analyze them.

The ovarian egg has its cytoplasm in a delicate superficial layer (blastema), and in a delicate intravitellar reticulum connecting this with the cytoplasmic mass around the adcentral nucleus. Doubtless there is some polarity of these parts, but I have not been able to discover it. At maturation the nucleus becomes placed at the surface, where it originates the first polar body; the second polar spindle then moves centrad, forming the second polar body at the boundary of the outer and inner yolk layers, and the egg nucleus (pronucleus) finally reaches the center again (cf. my paper of 1907). These movements are in all probability due to cytoplasmic currents. For it is doubtful whether rounded nuclei or mitotic spindles possess automatic movements; they seem to be transported by the cytoplasm. And the fluid yolk substance between the yolk globules is probably inert, as are certainly the globules, and such flowings as it may exhibit are probably induced by cytoplasmic influence.

The cleavage of the egg consists of movements leading to the transposition of all nuclei and cytoplasm to the surface. This also is a general cytoplasmic movement, of the nature of either a flowing or a contraction.

The later movements are in part upon the yolk surface; in part

they consist of movements of cells within the yolk, but they are blastodermic and not vitellar.

The early blastoderm consists of a peripheral layer of cells equally numerous at all points of its surface. The ventral germ disc became established by (1) more rapid multiplication of cells at that region, and (2) to less extent by migration of cells along the periphery toward that pole. When the germ disc is forming there appear for the first time cell membranes, while before this period the only cell membrane was the vitelline membrane, and the cytoplasm represented one great reticular syncytium.

During gastrulation there are two movements. First, a sinking of cells below the blastoderm in the region of the two cumuli and along the margins of the germ disc, this due to change in position of spindles at those regions. And, second, an active autonomous wandering of the cells (vitellocytes) so invaginated partly into the yolk, but mainly upon it. It is noteworthy that the wandering of these cells does not carry them into the extraembryonic area, therefore some chemotactic influence must hold them within the germinal area. The movement of the vitellocytes is amoeboid and, to some extent, independent of blastodermic changes. The mesoblastic and mesentoblastic elements are again produced by vertical mitoses, but their cells remain parts of the blastoderm.

The elongation of the germ disc is due to rapidly repeated mitoses in the region just anterior to the caudal lobe, and this is what occasions the dislocation of that lobe around the surface of the egg.

Coincident with this lengthening of the germ disc arise the protozonites by a segmental rearrangement of the previously continuous mesoblast, the latter dividing into successive transverse masses. This is not a result of the lengthening of the embryo, for the cephalothoracal protozonites appear after their region has attained its full length. It is rather an autonomous movement of the mesoblast itself, a dehiscence of its cells at serially repeated points. Neither ectoblast nor yolk cells share in this process. This movement seems to take place by mesoblast cells at certain points passing beneath their comrades to form the splanchnic layer; certain cells at the anterior border

of each protozonite appear to move caudad, and others at its posterior border to migrate cephalad, whereby transverse clefts come to subdivide the mesoblast. I could not positively ascertain whether this itself is due to change in position of cleavage spindles at certain points or to a creeping of cells. Immediately afterward each protozonite divides into a right and left somite, between which is the ventral nucleus lined only by ectoblast. This movement also is limited to the mesoblast, it marks the separation of a right and left half of the body, and seems to be directly due to automatic movement of mesoblast masses away from the midline. One of its immediate effects is the outgrowth of the appendages with their enlarging coelomic cavities. There is no likelihood that the ectoblast pulls out the mesoblast in the formation of the appendages, rather mesoblast must push out ectoblast. But whether the appendages are originated by outpushing of the somatic mesoblast itself or by movement of yolk fluid into the coelomic cavities is an open question.

After the first appearance of the appendages other changes occur, of which the first three are movements of the ectoblast that had up to this time remained rather passive. First, the ectoblast thickens right and left of the midline, forming the central nervous system. Unlike the mesoblast segments, the ganglia are paired from the start; this is due to vertical mitoses at segmentally separated paired points. Second, there is invagination of the stomodaeum and of other parts of the cephalic lobe (cerebral ganglia, anterior and posterior lateral vesicles); this is in part due to vertical position of the mitotic spindles, but equally to invagination of layers of cells engendered probably by mechanical pressure of other parts. Third, from the extraembryonic ectoblast arise by delamination the blood cells, and these gradually wander into the embryonic body probably by amoeboid movement. Lastly, there is gradual extension dorsad of the somites by increased cell division at their dorsal borders.

Summarizing the various movements just detailed, we find they all can be reduced to two groups of phenomena: (1) cytoplasmic movements, (2) interaction of cytoplasmic movements with external pressure and strains. Of the first group we find: (*a*) movements of the cytoplasm as a whole before cell membranes become established;

and (*b*) movements of particular cells or particular groups of cells after cell membranes have appeared. That is, the formation of relatively firm cell membranes inhibits or restricts movements of the cytoplasm as a whole, it reduces such movements to intracellular processes. Therefore, cell membranes are not only of importance in metabolism, acting as selective osmotic membranes, as the physiologists have proven, but their presence is likewise a factor in embryonic differentiation, for they divide the embryo into somewhat independent territories, each of which then pursues its own differentiation process more or less apart from the others. Conklin (1902) has most ably and minutely analyzed cell movements during development and resolved them into cytoplasmic flowings, and has shown how important a factor they are in differentiation; his observations were made upon the holoblastic egg of a Gasteropod. But I believe the modifying influence of the presence of cell membranes upon these movements has not been pointed out. Then, in the Spider's egg, the cytoplasmic movements after establishment of cell membranes are of two kinds: (1) intracellular movements leading to change in position of axes of mitotic spindles; and (2) cytoplasmic flowings in the rest stage that lead to amoeboid locomotion of single cells (as vitellocytes and blood cells), and to dehiscence, probably also by amoeboid movement, of masses of cells (as in the formation of the somites). Cells that exhibit such locomotion are in a sense the most autonomous and independent of all; and their locomotion may be referable to particular chemotactic influences, though it must be borne in mind that the idea "chemotaxis" is not explanatory, but is often evasive of the question at issue; it simply puts substance in the place of motion.

Finally, I would discuss one complex movement of the embryo as a whole, the reversion process, and we may first mention the explanations that have been given before. Claparède (1862) explained the process of reversion as accomplished by widening of the ventral sulcus bringing the bases of the legs to the dorsal side, while the movement of the tail lobe would be a consequence of this process. Barrois (1878) ascribed reversion to displacement of the anal segment producing modification in the angle of divergence of the abdominal "germ bands"; but he did not explain the cause of the

first movement of the caudal lobe. Balfour (1880) referred to "elongation of the dorsal region, *i. e.,* the region on the dorsal surface between the anal and procephalic lobes"; but he failed to account for such dorsal elongation. Locy (1886) held that there is a shortening of the ventral surface of the embryo which draws the tail-lobe forward, thus explaining how the apex of this lobe becomes separated from the surface of the embryo; "a shortening of the ventral band does take place, which is at least equal to the pre-existing tail-fold, and the tail is in consequence drawn forward ventrally." The cause of "the ventral movement of the passive yolk mass must be found in the relative pressures exerted upon it by the dorsal area, on the one hand, and the ventral area, on the other. * * * The principal force, then, that pulls the nerve bands away from the ventral surface is the one which tends to reduce the width (not as Balfour will, the one that increases the length) of the dorsal region. The evident cause for this reduction in width is the dorsal concentration of the ectodermic elements which accompanies the formation of the so-called terga, and this is also the cause for the descent of the yolk mass." Wallstabe has given the most detailed description of the external changes during reversion.

Each investigator who has tried to explain the mechanics of reversion has differed from his predecessor, and so must I. The process is a very complex one and can hardly be ascribed to any single factor. The first intimation of the process is the widening of the ventral sulcus, particularly in its middle portion (compare Fig. 74, Plate VI, with Fig. 78, Plate VII). This widening cannot be due to automatic growth of the ventral sulcus itself, for this is composed of a very thin plate of cells hardly capable of exerting a strong pressure upon the thicker and heavier regions of the embryo; this widening of the ventral sulcus is rather a result than a cause of the reversion. A pressure is exerted upon the yolk by the invagination of portion of the cephalic region (stomodaeum, ganglia, Figs. 89, 91a); this pressure would be transmitted through the yolk to the weak portion of the embryo, the ventral sulcus, and help to widen the latter. But such pressure alone would be incapable of producing the whole set of changes that take place. A more powerful factor is, probably, in-

crease in length of the thoracic limbs; these fairly rigid cylinders grow in length and thickness, press on the one hand against the strong chorion of the egg, on the other against the lateral walls of the thorax, and since these limbs are not capable of much bending they would press the lateral walls of the embryo dorsad. This pressure would tend to narrow the extraembryonic area and thereby to push the abdomen out of its dorsal position.

Further, the great widening of the ventral sulcus at the same time shortens this sulcus, and this shortening would exert a pull upon those margins of the abdomen bordering this sulcus. Throughout these changes the yolk mass behaves passively, and in *Theridium* does not produce a particularly prominent ventral sac. Thus, the main factor in reversion seems to be the lengthening of the thoracic appendages and the pressures they exert upon the lateral walls of the body.

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EXPLANATION OF PLATES I-VIII.

All the drawings are made with the camera lucida, and in the greater number the yolk has been represented by simple shading. The arrow on some of the figures points to the anterior (cephalic) pole. The following abbreviations have been employed:

Ab. 1-*Ab.* 8, Abdominal segments 1-8.
Ab. 2. *B-Ab.* 5, *B.*, limb buds of abdominal segments 2-5.
Ab. G. 1-*Ab. G.* 6, abdominal ganglia 1-6.
A. L. V., antero-lateral vesicle.
Bl., blood-cell.
Caud., caudal lobe (telson).
Cav., central fluid cavity within yolk.
Cav. B., outer boundary of this cavity.
Ce. G., cerebral ganglion.
Ceph., cephalic lobe.
Ce. R., cerebral ridge.
Chel., cheliceron.
Chel. Coel., cheliceral coelom.
Chel. G., cheliceral ganglion.
Chel. Mes., cheliceral mesoblast.
Coel., coelom.
Cum. A., anterior cumulus.
Cum. P., posterior cumulus.
Cyt., cytoplasm.
Ect., ectoblast.
Ent., definite entoblast.
Ex., extraembryonic blastoderm.
Fov., fovea semicircularis.
Gang., ganglion.
Gast., gastrocoel.
G. B., peripheral boundary of germ disc.
G. C.? germ cell.
H., heart.
L. 1-*L.* 4., ambulatory appendages (or their segments).

L. 1. G.-L. 4. G., ganglia of ambulatory appendages.
M. E., median eye.
Med. L., median line.
Mes., mesoblast.
Mes. E., mesentoblast.
M. G., midgut.
Opt. G., optic ganglion.
Ped., pedipalp.
Ped. G., pedipalpal ganglion.
Ped M., maxillary plate of pedipalp.
P. L. V., postero-lateral vesicle.
Pr., prominence of antero-lateral vesicle.
Pul. C., invagination cavity of lung book.
Pul. L., lamellae of lung book.
R. Coel., rostral coelom.
R. Mes., rostral mesoblast.
Ros., rostrum.
So. Mes., somatic mesoblast.
Sp., accessory sperm nuclei.
Sp. Mes., splanchnic mesoblast.
Sto., stomodaeum.
Sto. L., lateral lip of stomodaeum.
Sul. A., sulcus anterior.
Sul. v., ventral sulcus.
Th. Ab., boundary of thorax and abdomen.
Vit., vitellus (yolk).
Vit. C., vitellocyte (yolk cell).

PLATE I.

Fig. 1.—Section of egg of 5 hours, 2-cell stage, only a portion of cytoplasmic meshwork shown. × 142.

Fig. 2.—Segment of section of egg, 5¼ hours, early 4-cell stage, meshwork of cytoplasm not shown. × 142.

Fig. 3.—Composite drawing from sections of egg of 7½ hours, 8-cell stage, yolk not shown. × 142.

Fig. 4.—Segment of one section of the preceding egg. × 142.

Fig. 5.—Composite drawing from section of egg of ca. 10½ hours, 16-cell stage, yolk not shown, peripheral layer only of cytoplasm reproduced. × 142.

Fig. 6.—Segment of one section of preceding egg. × 142.

Fig. 7.—Composite drawing from section of egg of 11 hours, about 32-cell stage, yolk not shown, peripheral layer only of cytoplasm reproduced. × 142.

Fig. 8.—Surface view of one hemisphere of an egg of 12 hours, stage of 60-70 cells; few of the cells have reached the superficial layer of cytoplasm. × 142.

Fig. 9.—Segment of a section of an egg of the stage of Fig. 10. × 142.

Fig. 10.—Surface view of one hemisphere of an egg of 11½ hours, stage of ca. 130-140 cells in superficial position. × 142.

Fig. 11.—Similar view of an egg of 14 hours, stage of ca. 300 cells. × 142.

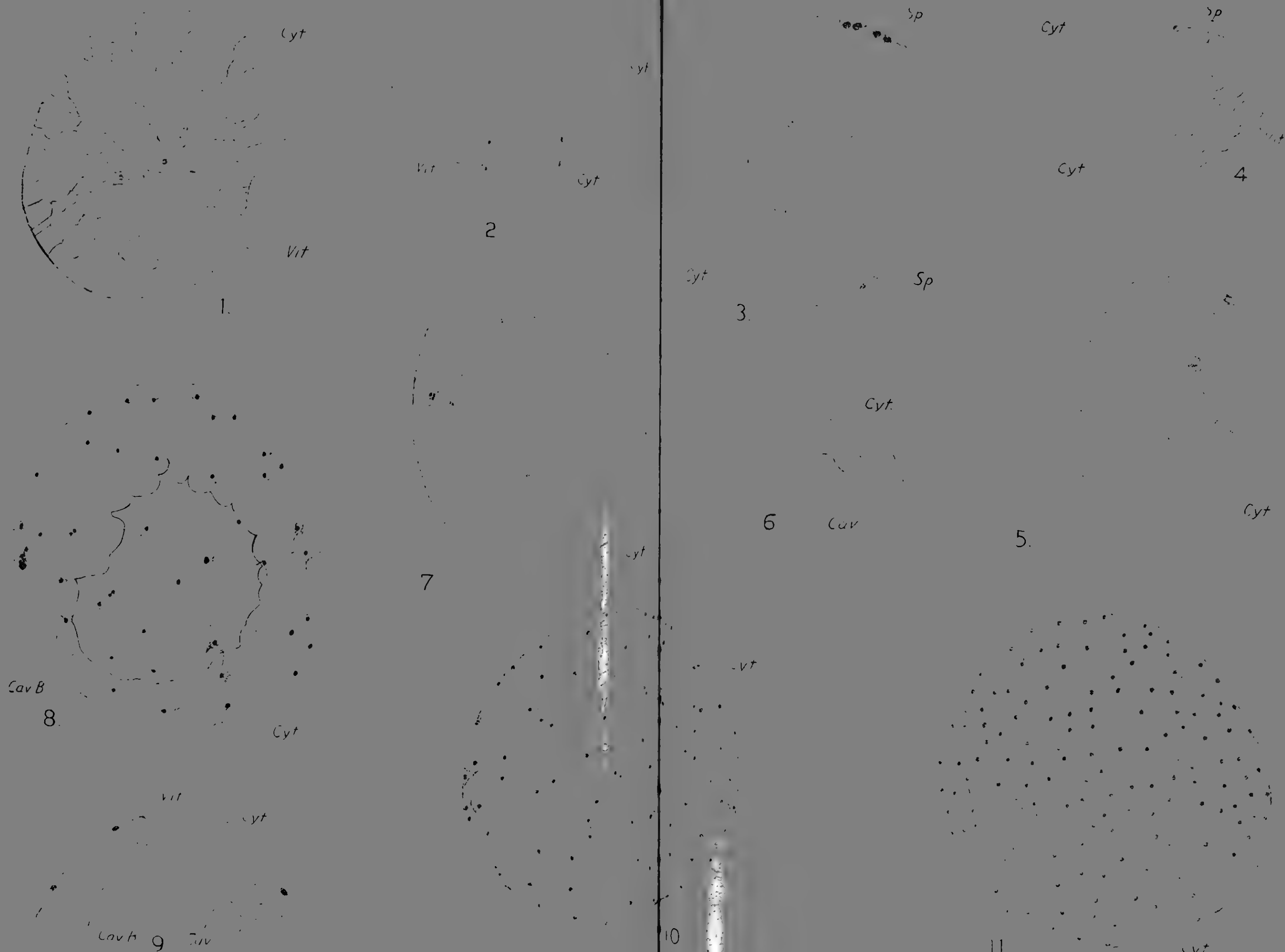
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PLATE II.

Fig. 12.—Surface view of one side of egg of 19½ hours, 173 cells shown on this surface. × 142.

Fig. 13.—Portion of section through an egg of the same stage as the preceding, showing the ventral pole. × 142.

Fig. 14.—Section through anterior cumulus of an egg of 21 hours, only 2 cells invaginated. × 360.

Fig. 15.—Surface view of ventral pole of an egg of 24 hours, 417 cells shown on this surface, at the lower part of the figure the cells appear most crowded because that portion of the germ disc is most convex. The cells have short intercellular processes that are not drawn. × 142.

Fig. 16.—Somewhat oblique cross section of egg of 24 hours, through the anterior cumulus, less than 12 cells invaginated. × 142.

Fig. 17.—Somewhat oblique cross section through anterior cumulus of egg of 24 hours, about 8 cells invaginated. × 360.

Fig. 18.—Ventral view of unstained germ disc seen in alcohol, 22 hours. × 30.

Fig. 19.—Cross section of anterior cumulus of an egg of 22 hours, 12-13 cells invaginated. × 360.

Fig. 20.—Cross section of an egg of ca. 26 hours. × 142.

Fig. 21.—Cross section through anterior cumulus of an egg of ca. 26 hours, about 25 cells invaginated. × 360.

Fig. 22.—Somewhat oblique ventral view of germ disc of 28 hours, somewhat more than 500 cells shown. × 142.

Fig. 23.—Surface view of unstained germ disc of 30 hours, seen in alcohol. × 30.

Fig. 24.—Cross section of anterior cumulus of an egg of 30 hours. × 360.

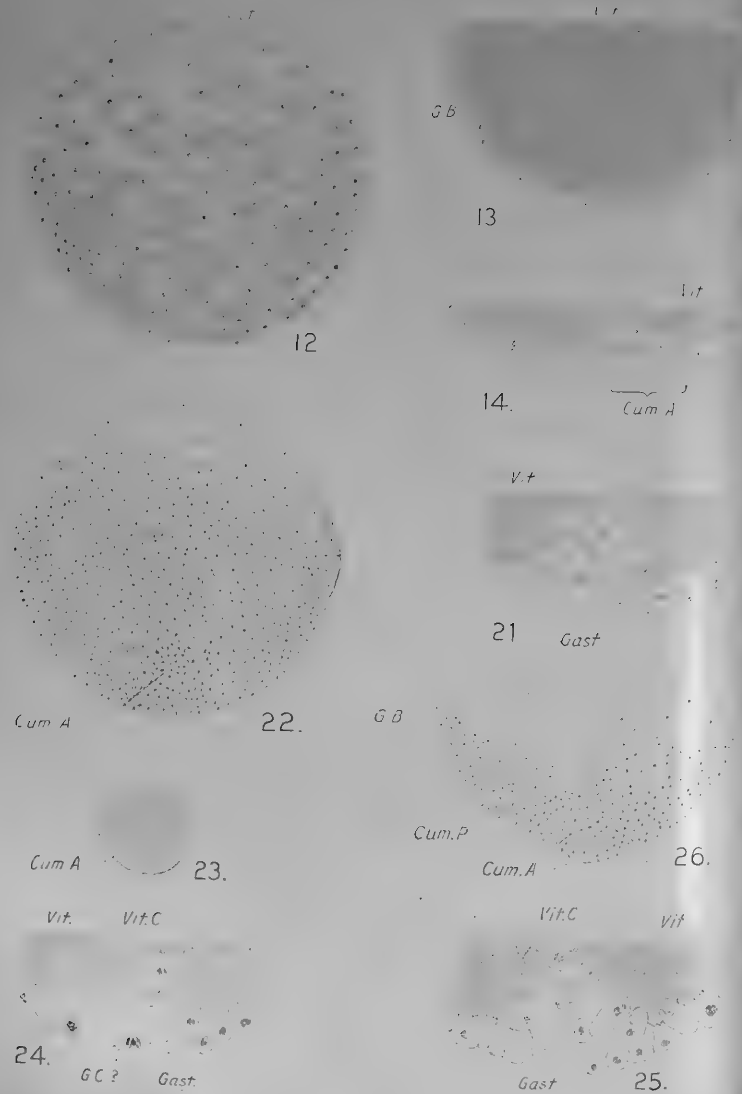
Fig. 25.—Oblique cross section of anterior cumulus, egg of 30 hours. × 360.

Fig. 26.—Surface latero-ventral view of germ disc, of 30½ hours, with two artificial breaks in its side; the inner bounding line of each cumulus shows how deep its cells project into the yolk; 268 cells shown. × 142.

Fig. 27.—Median section through both cumuli of germ disc, 30½ hours. × 142.

Fig. 28.—Median section through anterior cumulus, 30½ hours. × 360.

Fig. 29.—Surface view of unstained germ disc, seen in alcohol, 54 hours (same stage as Fig. 30). × 30.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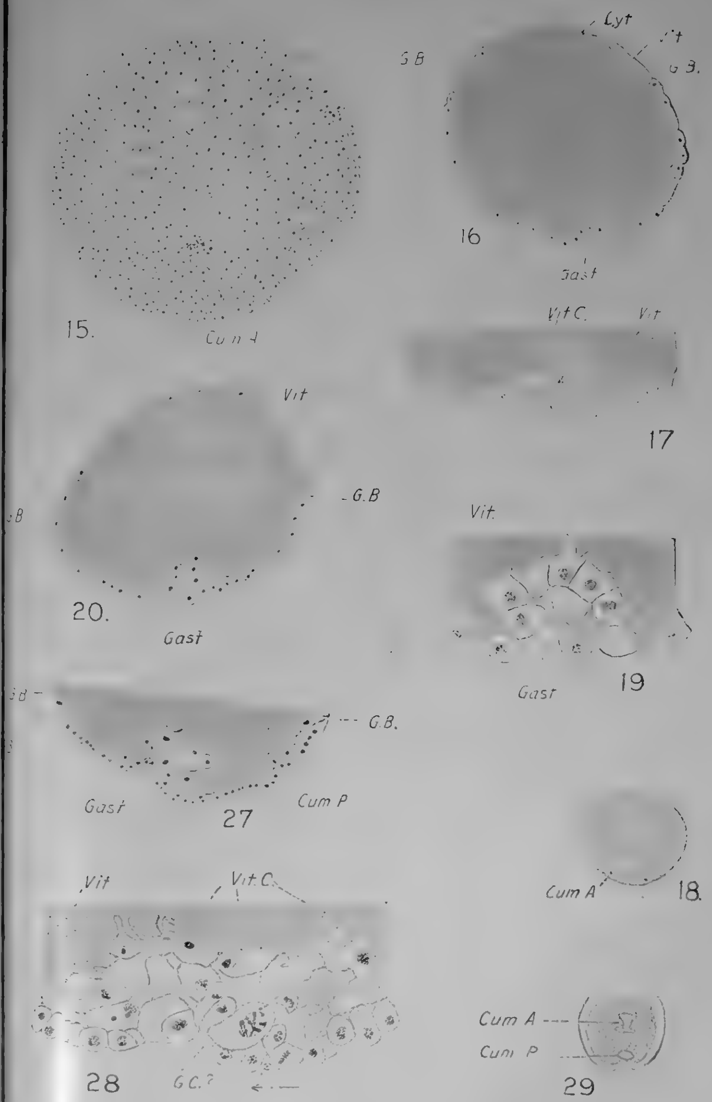
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G B
Cyt
G B.
16
Gast
Vit C.
Vit
17
Vit
G.B
20.
Gast
Vit.
Gast
19
G.B.
Gast
27
Cum P
Cum A
18.
Vit
Vit.C.
28
G C.?
Cum A
Cum P
29

PLATE III.

FIGS. 30, A, B.—Oblique longitudinal section, of germ disc of ca. 54 hours, A, through posterior cumulus (but to one side of anterior cumulus), B, lateral from both cumuli. × 142.

FIG. 31.—Cross section through middle of germ disc of egg of ca. 54 hours; vitellocytes forming from anterior cumulus and from lateral marginal germ disc. × 142.

FIG. 32.—Surface view of germ disc of ca. 54 hours, seen in alcohol. × 30.

FIG. 33.—Similar view of germ disc of ca. 50 hours. × 30.

FIG. 34.—Ventral surface view of egg of ca. 37 hours, the shaded area marking the region of axial cell invagination; 1222 cells on surface of the germ disc. × 142.

FIG. 35.—Cross section of posterior cumulus ca. 37 hours. × 360.

FIG. 36.—Longitudinal section of posterior cumulus ca. 37 hours. × 360.

FIGS. 37 A. B.—Oblique cross sections of anterior cumulus of an egg of ca. 37 hours. × 360.

FIG. 38 A.—Oblique longitudinal section of germ disc through the anterior cumulus, ca. 37 hours. × 142.

FIG. 38 B.—Section of the anterior cumulus of the egg shown in the preceding figure, drawn to larger scale. × 360.

FIG. 39.—Cross section of germ disc at anterior edge of anterior cumulus, ca. 37 hours. × 142.

FIGS. 40 A, B.—Ventral surface views of a germ disc of ca. 49 hours; A, shows the nuclei of the superficial cells (1441 in number); and B, the nuclei of the larger cells (69 in number) that have sunk below the former and spread out upon the surface of the yolk. × 142.

FIG. 41 A.—Exact median section of an egg of ca. 49 hours. × 142.

FIGS. 41 B, C.—Two other longitudinal sections of the preceding egg, both in the region of the anterior cumulus. × 360.

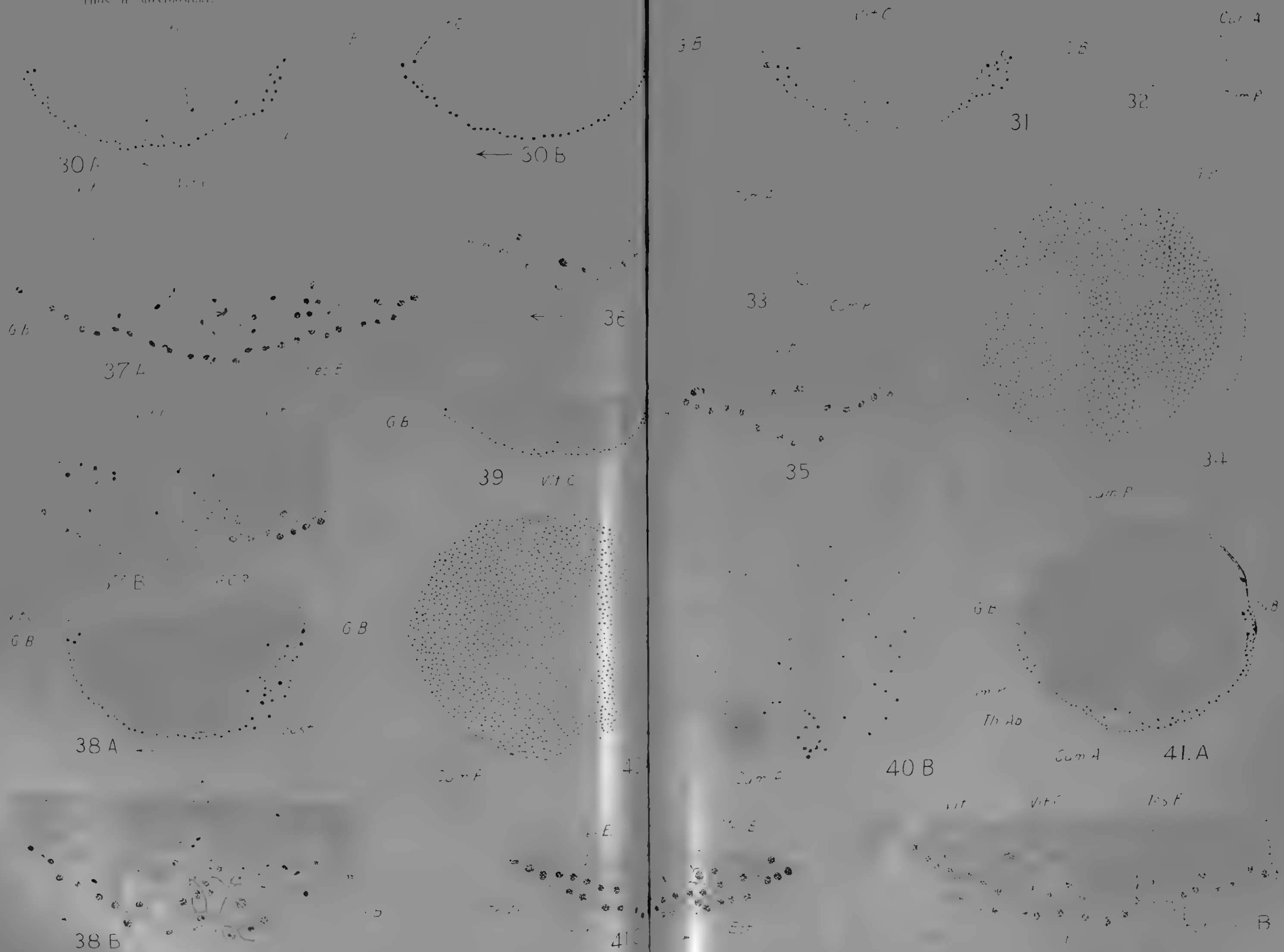


PLATE IV.

Fig. 42 A.—Median section of germ disc of ca. 49 hours. × 142.

Fig. 42 B.—Posterior portion of the same section. × 360.

Figs. 43 A-C.—Portions of cross sections of an egg of ca. 49 hours; A, in midline anterior to anterior cumulus; B, in lateral region; C, at posterior cumulus. × 360.

Fig. 44.—Surface view of unstained germ disc of ca. 49 hours, seen in alcohol. × 30.

Fig. 45.—Ventral view of germ disc with 4 protozonites, 60¼ hours. × 60.

Fig. 46.—Ventral view of germ disc with 5 protozonites, 60 hours. × 60.

Fig. 47.—Lateral view of embryo with 5 protozonites, 60 hours. × 30.

Fig. 48.—Oblique longitudinal section of embryo with 5 protozonites, 60 hours. × 142.

Figs. 49 A, B.—Portions of cross sections of an embryo of 60¼ hours; A, through extraembryonic area; B, through half of the germ disc in the plane of a protozonite. × 360.

Figs. 50 A-C.—Portions of median sections of germ disc of 60¼ hours. A, cephalic lobe region; B, caudal lobe region; C, through two of the anterior protozonites. × 360.

Fig. 51.—Oblique latero-ventral surface view, 60¼ hours, first appearance of appendages. × 60.

Fig. 52.—Oblique latero-ventral surface view, ca. 62 hours. × 60.

Fig. 53.—Lateral surface view, ca. 62 hours. × 60.

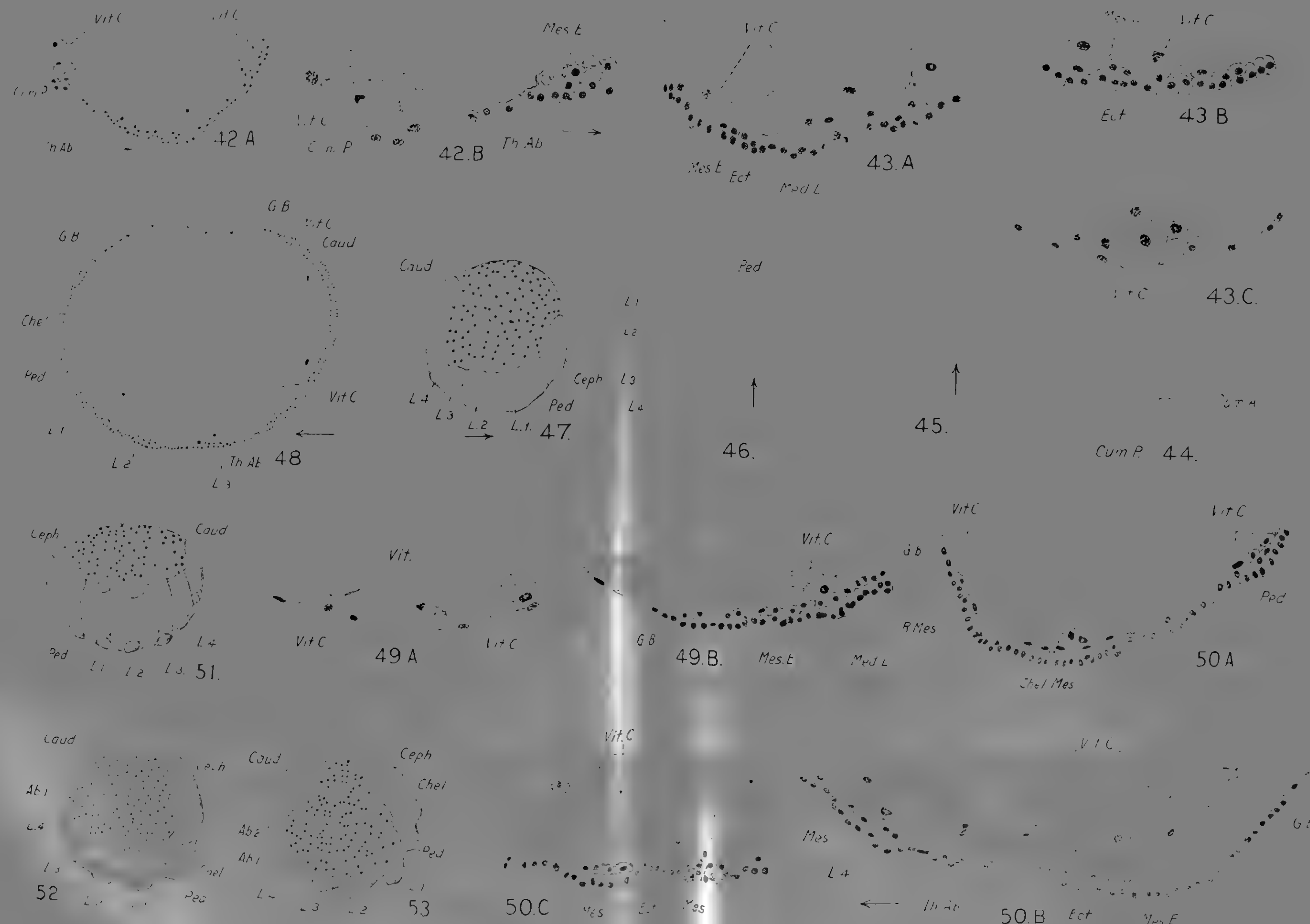

42 A
42.B
43.A
43 B
43.C.
44.
45.
46.
47.
48
49 A
49.B.
50 A
50.B
50.C
51.
52
53
Vit C
Th Ab
Mes E
Ect
Med L
Cum P
G B
Caud
Chel
Ped
Ceph
L 1
L 2
L 3
L 4
Vit.
Mes.E
R Mes
Chel Mes
Mes
Ab 1
Ab 2

PLATE V.

Fig. 54.—Cross section of caudal lobe in it's posterior half, ca. 62 hours. × 360.

Fig. 55.—Portion of cross section through third or fourth pair of thoracal limbs, ca. 62 hours. × 360.

Figs. 56 A, B.—Median section of posterior region of embryo of ca. 62 hours, the posterior end of the caudal lobe represented in B continuous with the right end of the portion of the section of A. × 360.

Fig. 56 C.—Median section of cephalic region of the embryo shown in Figs. 56 A, B. × 360.

Fig. 56 D.—Longitudinal section of pedipalp of the same embryo. × 360.

Fig. 57.—Lateral surface view, 73 hours. × 60.

Fig. 58.—Oblique dorso-lateral view, ca. 75 hours. × 60.

Fig. 59.—Oblique antero-lateral surface view, ca. 75 hours. × 60.

Fig. 60 A.—Longitudinal section of the region where cephalic and caudal lobes meet, 73 hours. × 360.

Fig. 60 B.—Longitudinal section of portion of head lobe, cheliceron and pedipalp, of the same embryo. × 360.

Fig. 61.—Cross section of a little more than one-half of an abdominal segment, ca. 75 hours. × 360.

Fig. 62.—Portion of cross section of thorax, showing one limb and its ganglion, ca. 75 hours. × 360.

Fig. 63.—Lateral surface view, ca. 86½ hours. × 60.

Fig. 64.—Oblique dorso-anterior surface view, ca. 86½ hours. × 60.

Fig. 65.—Oblique ventral surface view of cephalothorax, ca. 86½ hours. × 60.

Fig. 66.—Ventral view of abdomen, ca. 86½ hours. × 60.


54
55
56.A
56.B.
56 C
56.D
57
58
59.
60 A
60.B.
61
62
63.
64
65
66

PLATE VI.

FIG. 67 A.—Somewhat oblique longitudinal section of cephalic region, ca. 86½ hours. × 360.

FIG. 67 B.—Longitudinal section of the same embryo through the lateral edge of the stomodaeum. × 360.

FIG. 67 C.—Longitudinal section through extraembryonic blood-forming area of the same stage. × 720.

FIGS. 68 A-C.—Transverse sections of cephalic lobe of embryo of 86½ hours; A, through rostral extremities; B, through stomodaeum; C, through antero-lateral vesicle in the same plane as Fig. A. × 360.

FIG. 68 D.—Transverse section of the same embryo, the rostral appendages above, a pair of thoracal legs below. × 142.

FIG. 68 E.—Portion of left half of preceding drawing, on higher scale. × 360.

FIG. 68. F.—Cross section of same embryo, cephalic lobe anterior to the rostrum. × 142.

FIG. 68 G.—Cross section of the same embryo, lateral margin of head lobe and blood-forming region of extraembryonic area. × 720.

FIG. 69.—Longitudinal section through abdomen, 86½ hours. × 360.

FIG. 70.—Longitudinal section of the fourth leg and first abdominal segment, 86½ hours. × 360.

FIG. 71.—Oblique antero-lateral surface view, ca. 97 hours. × 60.

FIG. 72.—Anterior surface view, ca. 97 hours. × 60.

FIG. 73.—Latero-posterior surface view, ca. 97 hours. × 60.

FIG. 74.—Ventro-posterior surface view, ca. 97 hours. × 60.

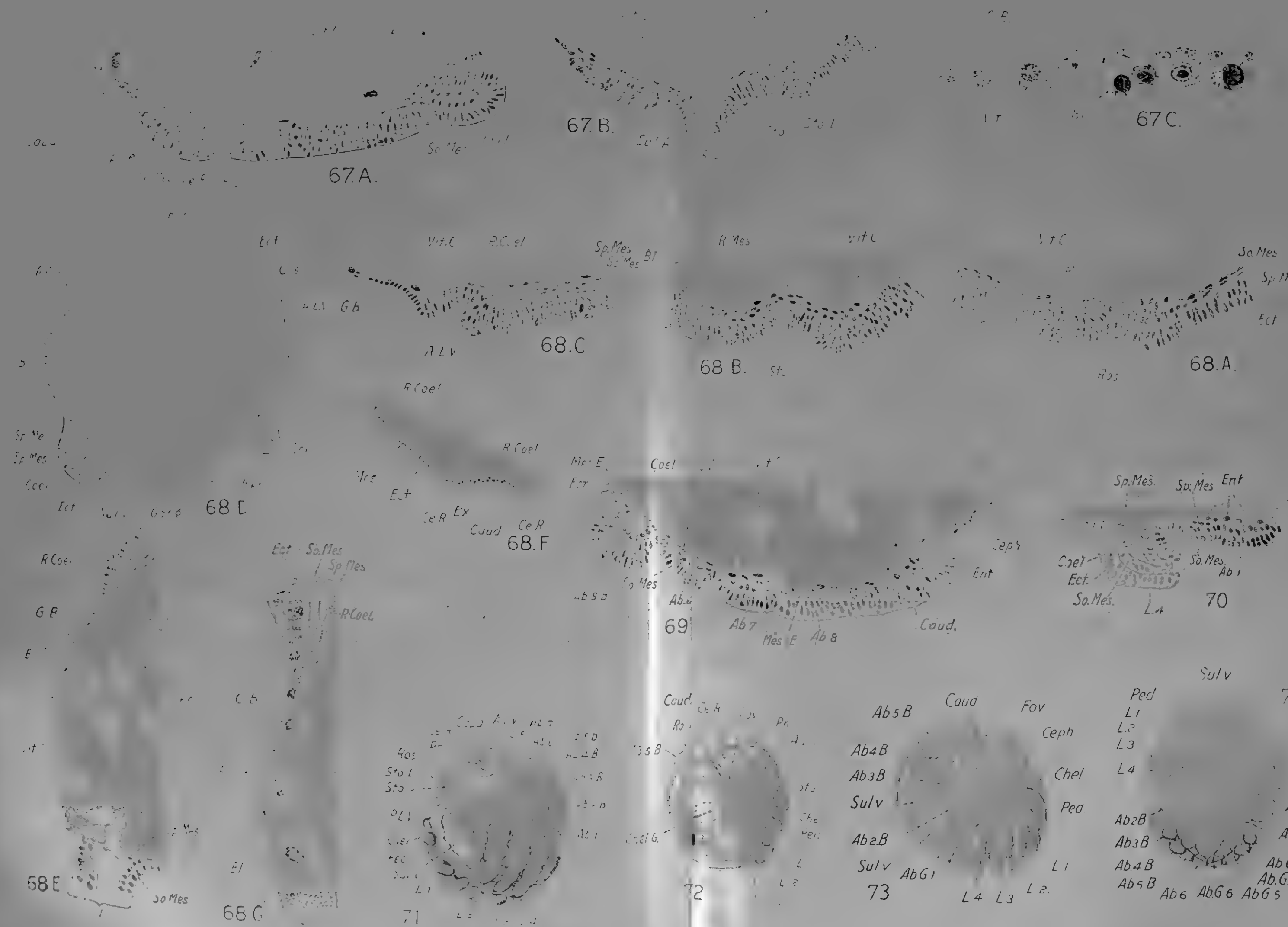

67.A.
67.B.
67.C.
68.A.
68.B.
68.C
68.D
68.E
68.F
68.G
69
70
71
72
73
74
Sp.Mes
So.Mes
Ect
Ent
Coel
R.Coel
Caud.
Ab 7
Ab 8
Mes.E
Ceph
Fov
Chel
Ped.
Sulv
L 1
L 2
L 3
L 4
Ab 5 B
Ab 4 B
Ab 3 B
Ab 2 B
Ab 6
AbG 1
AbG 2
AbG 3.
AbG 4
AbG 5
AbG 6

PLATE VII.

FIG. 75 A.—Somewhat oblique longitudinal section of posterior portion of abdomen, ca. 97 hours. × 360.

FIG. 75 B.—Similar section of head region and caudal lobe of the same embryo. × 142.

FIGS. 76 A-C.—Cross sections of embryo of ca. 97 hours; A, through head lobe anterior to rostrum; B, through stomodaeum; C, through chelicera. × 142.

FIG. 77.—Cross secction of abdomen in plane of the fifth segment, ca. 97 hours. × 360.

FIG. 78.—Postero-ventral surface view, ca. 108 hours. × 60.

FIG. 79.—Antero-ventral surface view, ca. 108 hours. × 60.

FIGS. 80 A-E.—Oblique longitudinal sections of an embryo of ca. 108 hours; A, through cheliceron and postero-lateral vesicle. × 360. B, through stomodaeum. × 142. C, through antero-lateral vesicle. × 142. D, through fovea and lateral wall of stomodaeum. × 142. E, through posterior end of abdomen. × 360.

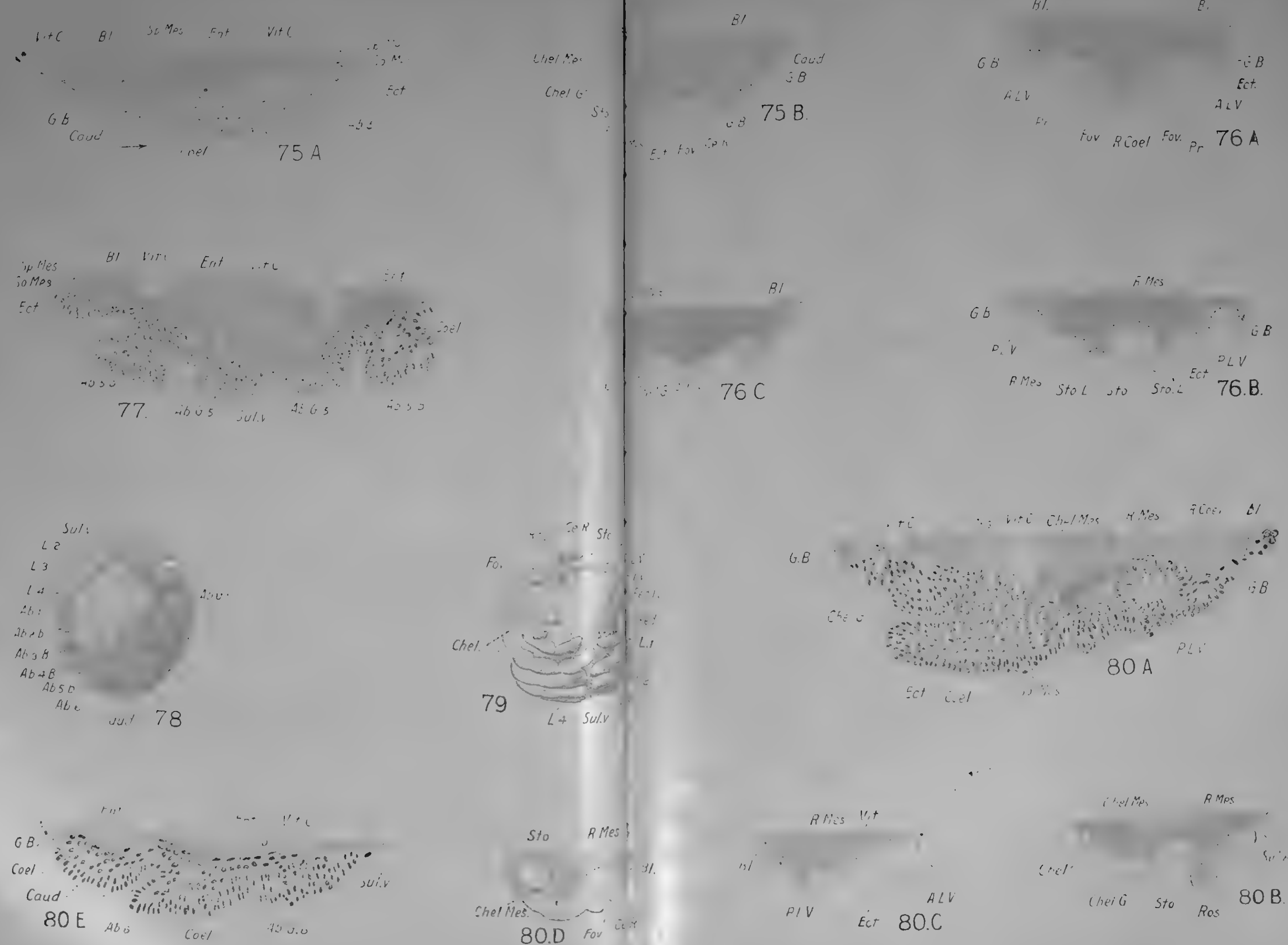

75 A
75 B.
76 A
77.
76 C
76.B.
78
79
80 A
80 E
80.D
80.C
80 B.

PLATE VIII.

FIG. 81.—Oblique ventro-posterior surface view, ca. 98 hours. × 60.

FIG. 82.—Ventral surface view, ca. 98 hours. × 60.

FIG. 83.—Cephalic surface view, 120 hours. × 60.

FIG. 84.—Oblique lateral surface view, ca. 98 hours. × 60.

FIG. 85.—Dorsal surface view, ca. 98 hours. × 60.

FIG. 86.—Oblique dorso-posterior view, ca. 127 hours. × 60.

FIG. 87 A.—Oblique cross section of cephalic region, ca. 98 hours. × 142.

FIG. 87 B.—Oblique cross section of the same embryo, on the right in the plane of a cheliceron, on the left anterior to the cheliceron. × 142.

FIG. 87 C.—Oblique cross section of the caudal lobe of the same embryo. × 360.

FIG. 87 D.—Oblique cross-section of the heart (dorsal) region of the same embryo; the left-hand edge of the figure is near the base of a thoracal limb. × 360.

FIG. 88.—Cross section of heart region, ca., 98 hours. × 360.

FIG. 89.—Longitudinal section of an embryo of ca. 127 hours. × 142.

FIG. 90.—Oblique longitudinal section through cephalic region, ca. 98 hours. × 142.

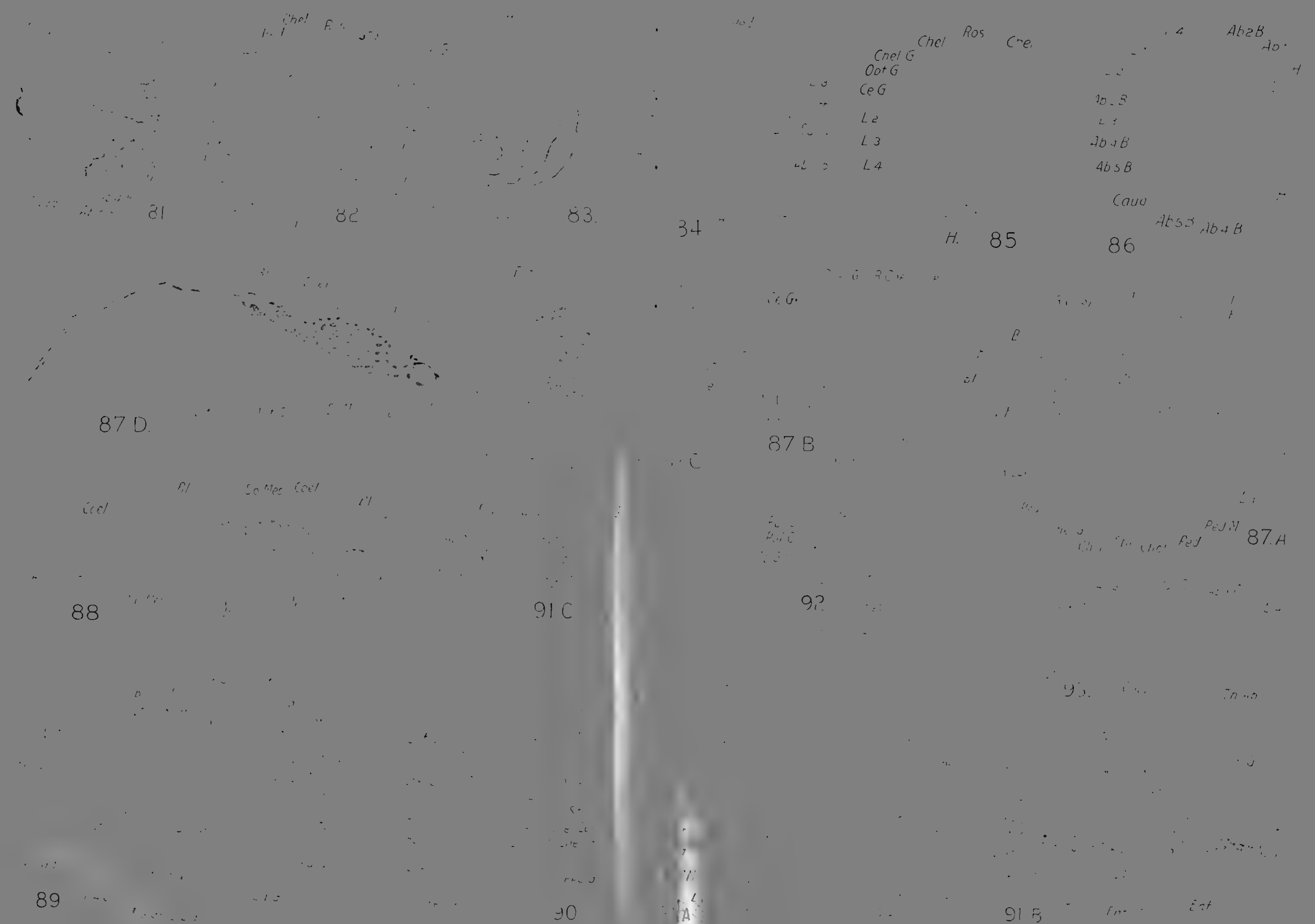
FIG. 91 A.—Longitudinal section, ca, 98 hours. × 142.

FIG. 91 B.—Longitudinal section of caudal lobe of the same embryo. × 360.

FIG. 91 C.—Longitudinal section of lung book region of the same embryo. × 360.

FIG. 92.—Longitudinal section of lung book cut to one side of its mesoblast sac, ca. 127 hours. × 360.

FIG. 93.—Section through the posterior thoracal and the first four abdominal segments, 120 hours. × 142.


81
82
83.
84
Chel
Ros
Chel G
Opt G
Ce G
L 2
L 3
L 4
H.
85
Ab 2 B
Ab 4 B
Ab 5 B
Caud
86
87 D.
87 B
87 A
88
91 C
92
95.
89
90
91 B

OBSERVATIONS ON OOKINESIS IN *CEREBRATULUS LACTEUS*, VERRILL.[1]

NAOHIDE YATSU.

CONTENTS.

[1]I wish to express my obligation to Professor Wilson for his advice and suggestion during the progress of this work and the preparation of this paper, and to Professor Kingsley, Director of the Harpswell Laboratory, for his continued kindness during my stay at his laboratory.

I. Introduction.

It is nine years since Coe ('99) wrote his excellent paper on the maturation and fertilization of the egg of *Cerebratulus marginatus* Renier. The remarkable advance of our knowledge of the achromatic structure of the cell attained during this time has made it desirable to re-examine some of the most important phenomena in the nemertine egg; for it is one of the most favorable objects for cytological study. Five years ago Professor Wilson suggested to me to make a careful study of maturation, fertilization and early cleavage stages of the egg of *Cerebratulus lacteus,* partly in order to clear up some disputed cytological problems and partly to give a morphological basis for my work on experimental cytology and embryology.

The material, from which the results of the present paper were obtained, consists of several complete series of the early developmental stages of the egg of *Cerebratulus lacteus* put up by Professor Wilson and Dr. Sutton in the summer of 1902, at South Harpswell, Maine. A few lots treated with some salt solutions were fixed by the writer, and were made use of for the sake of comparison. To supplement the general discussion a few results obtained from the study of the egg of *Asterias forbesii* have been incorporated.[2]

II. Structure of the Egg.

(a) *Cytoplasm.*

The cytoplasm of the egg of *C. lacteus,* as in many other forms, shows an alveolar structure, consisting of fine yolk drops of fairly

[2]I am under great obligation to Dr. H. M. Smith and other members of the Biological Laboratory of the United States Fish Commission at Wood's Hole, where my cytological work on the starfish egg was partly carried out in the summer of 1902.

constant size suspended in hyaloplasm.[3] The former can readily be seen in the living egg, while the latter can be observed only in sections stained either with Delafield's hæmatoxylin (which stains hyaloplasm only) or with iron-hæmatoxylin, thoroughly extracted, and erythrosin as a counter-stain. Very young eggs stain dark, since they are made up almost entirely of hyaloplasm (cf. Wilson '99, p. 11). As I have not been able to make out any space between the yolk drop and hyaloplasm, the yolk drop itself represents alveolar substance, and is not contained in a distinct alveolar substance as Coe maintains ('99, p. 434). The yolk drop of the nemertine egg has, I think, a much greater power of withstanding the action of acetic-sublimate than that of the echinoderm egg, in which, under the same treatment, the alveolar substance is found completely dissolved. It is extremely difficult to determine the relative viscosity of the yolk drops and hyaloplasm. The latter, however, seems to me more fluid than the former, for the reason that, when the egg is crushed, the hyaloplasm flows out more readily than the yolk drops (Mrs. Andrews, '97, p. 82; Wilson, '99, p. 7, and his Pl. I, Fig. 7), and the polocytes do not contain in our case any yolk drops at all.

In the egg prior to the dissolution of the germinal vesicle one observes very readily, in sections as well as in the living state, that the yolk drops are disposed radially (Pl. I, Fig. 1) (cf. Bambeke, '98, Fig. 7, Pl. 25, p. 537; Giardina, '02, pp. 564-565; Gerould, '06, p. 82). This arrangement may indicate some nuclear activity upon the cytoplasm. After the germinal vesicle has faded this is disturbed by the mixture of the nuclear fluids and cytoplasm; some of the yolk drops pass into the nuclear area, while the nuclear fluid flows out into the cytoplasm. Thus the cytoplasm becomes richer in hyaloplasm even as far as the periphery, the yolk granules being less crowded than they were before. The amount of hyaloplasm in

[3]In the living egg of echinoderms Mathews observed that the cytoplasm is not alveolar but granular ('06). The term "alveole" has not been understood as a "hole," as he defines it, (p. 143), and its contents, the alveolar substance, differs markedly in the degree of viscosity ranging from thin watery drops to highly viscous yolk granules, (cf. Mrs. Andrews, '97, p. 14). The term alveolar may, therefore, be applied to the echinoderm egg without causing any inconvenience.

the cytoplasm is consequently much greater than is needed to maintain the alveolar structure. The cytoplasmic maturation of Delage, ('01), may mean that the cytoplasm becomes overloaded with the hyaloplasm. This is a favorable, if not absolutely necessary, condition for the formation of astral rays as correctly recognized by Mathews ('07, p. 97). In this connection one interesting fact may be mentioned, that is, before the dissolution of the germinal vesicle the spermatozoon remains as such in the egg, either the formation of the sperm aster or the swelling of the sperm head being suspended (Fol, '79). Ziegler observed in the egg of a nematode that the spermatozoon degenerates, when it enters an enucleated fragment from the egg with the germinal vesicle intact ('95, p. 363). Flemming's observation ('91) that the dividing cells of the salamander epithelium stain darker than the resting ones may show that before the reconstruction of the nucleus the cytoplasm is richer in hyaloplasm.[4]

(*b*) *Nuclear Fluid.*

In the living egg the nuclear fluid appears as a homogeneous liquid, not an emulsion of the different fluids as in the cytoplasm. Neither reticular nor alveolar structure is visible. In sections, however, the nuclear substance gives an entirely different aspect from what is seen in the living state. As is shown in Fig. 10 (Pl. I) the germinal vesicle is traversed by an irregular network. The meshes are not complete; many free ends of the branches can be observed. The apparent reticular structure of the nuclear fluid is, I think, simply an artifact produced by coagulation. Although there are many cases in which the nucleus actually contains reticulum or alveoles, *e. g.*, in the protozoan nucleus, yet in a good many cases the homogeneity of the nuclear fluid has not been correctly recognized (cf. Henneguy, '96, p. 106, Rhumbler, '96).

It is interesting to note that the nuclear fluid changes its nature the moment the nucleus begins to fade (cf. Lillie, '06, p. 166). Fig. 2 and 3 (Pl. I) show this relation very clearly. The nuclear fluid

[4]Throughout the present paper the figures are from the preparations of *Cerebratulus lacteus*, unless otherwise mentioned.

is now found precipitated in the form of flocculent masses, as though another kind of proteid had entered the nuclear area, and had mixed with the nuclear fluid. The precipitated granules are of fairly large size. They take a strong hæmatoxylin stain, and in this respect they resemble chromatin (basichromatin). Yet that they do not contain any basichromatin at all, is demonstrated by Auerbach's method, with which they take a strong fuchsin stain. There are several instances in which a chromatin diminution is said to take place at the formation of the first maturation figure (Wilson and Mathews, '95; Gardiner, '98; Griffin, '99; Coe, '99; Conklin, '02). But in these cases the writers may have taken the precipitated nuclear fluid for chromatin, simply because it is stained with hæmatoxylin. This phenomenon, I think, can hardly be called chromatin diminution.

The mass of precipitated nuclear fluid (residual substance of germinal vesicle, Lillie, '06) moves towards the animal pole taking a columnar shape around the first maturation figure (Pl. IV, Fig. 59). In the eggs kept unfertilized for several hours this plasm spreads out at the animal region, forming a layer thick at the middle and thinning towards the periphery (Kostanecki and Wierzejski, '96, p. 370; Kostanecki, '02, p. 272; Wilson, '03, p. 446, foot-note, Yatsu, '04, p. 134). In the cleavage stages the precipitated nuclear fluid, which is found outside the spindle at the metaphase, is absorbed to the equatorial plane at the anaphases (Text Fig. C, 1 and 2). The portion of the fibres included within this plasm is thicker and stains darker than the rest. This nuclear plasm seems to play an important *rôle* in the formation of the diastem (*vide infra*) and the midbody. I can, however, find no evidence that it is directly converted into the cell membrane as Rhumbler maintains ('97, pp. 696-697; '98, pp. 549-552; '99, p. 200).

It is noteworthy that at the prophase of the cleavage mitoses the rays which grow towards the nucleus from the astral centres (the rays which give rise to both the spindle and the chromosomal fibres) are after the dissolution of the nuclear walls, composed of two portions; namely, an extranuclear and an intranuclear part. The latter are thicker and stain more deeply (Pl. I, Fig. 4). The similar dif-

ference between these two portions was recently noticed by Bonnevie ('06, p. 282). This seems to show on the one hand that the nuclear fluid contributes to the growth of rays (cf. Mark, '81, p. 537), and on the other that a ray may be formed in the nonalveolar plasm (*vide infra*). The seeming pushing-in of the nuclear walls at the poles may indicate a passing out of the nuclear fluid, at the expense of which the rays grow.

The nuclear fluid after the dissolution of the nuclear membrane has a strong resemblance to hyaloplasm, both in its staining reaction and in the power of producing rays. While the nuclear fluid is not as a whole identical with the hyaloplasm, one may say in a general way that the nucleus is a storehouse of hyaloplasm.

(*c*) *Nucleolus.*

In the germinal vesicle three elements can be seen (Pl. I, Fig.1); a large plasmosome (principal nucleolus), smaller peripheral plasmosomes (accessory nucleoli[5]) and chromatin masses.

The larger plasmosome is usually single, seldom two to four are present (Pl. I, Figs. 1 and 8), and is situated in most cases half way between the nuclear membrane and the centre of the germinal vesicle. In the living state the plasmosome is a drop consisting in most cases of two portions; one a lenticular or crescentic refractive part, with a reddish tint suggesting the contractile vacuole of some ciliates, and the other a watery part, or "vacuole." The latter is rarely wanting.

In sections only the denser part comes into view as a solid body, the thinner part being represented either as a clear space containing irregular precipitated masses having the form of small discs resembling mammalian-blood corpuscles (Pl. I, Fig. 6), or entangled threads which stain green with bleu de lyon (Pl. I, Fig. 8). The denser part is usually lenticular or spherical, but sometimes it gives the appearance of a basket (Pl. I, Fig. 7). It is homogeneous and takes a deep plasma stain—dark yellowish green with Delafield's

[5]According to Flemming ('82, p. 146) the terminology is based simply on size. not on chemical nature.

hæmatoxylin or iron-hæmatoxylin. After the germinal vesicle has faded the denser part becomes a hollow sphere (Pl. I, Figs. 12, 14; Pl. IV, Fig. 60). It usually disappears at a late prophase of the first maturation mitosis, although sometimes it may be seen as late as the metaphase, it being taken up into the equatorial plate like the chromosomes.

The smaller plasmosomes vary in number, usually three or four, sometimes as many as nine (Pl. I, Fig. 1). These nucleoli are situated just beneath the nuclear membrane. They are about one-fifth of the larger in diameter, and sometimes are composed of denser and thinner parts as in the larger one (Pl. I, Fig. 11). They gradually dwindle (Pl. I, Fig. 10) and, when the germinal vesicle has faded, no trace of them can be found.

A chromatin mass, composed of chromatin spherules, is always found close by each of the smaller nucleoli and three or four of them are associated with the larger one. Sometimes one or two chromatin spherules are found imbedded in the latter (Pl. I, Fig. 12).[6]

The chromatin mass stains purple with Auerbach's fluid. At the beginning of the maturation, when the nuclear membrane is fading, *pari passu* with the dwindling of the smaller nucleoli, the chromatin masses resolve into smaller spherules. They become greener and greener when stained with Auerbach's fluid. After the smaller nucleoli have completely disappeared there are found some two dozen of chromatin granules of irregular shape staining brilliant green with the above fluid (Pl. I, Fig. 14). The number of the chromatin blocks is not constant. Of these only eighteen (Pl. I, Fig. 15) go to the equatorial place of the first maturation figure and become the definite chromosomes. The rest of the granules disappear without giving rise to the chromosomes (chromatin diminution).[7] The rest of

[6]In the egg treated with a solution of $CaCl_2$ (Yatsu, '05, p. 290) the chromosomes arise in the germinal vesicle as fine threads resembling those found in the segmentation nucleus (Pl. I. Fig. 13) (cf. Wilson '07a, pp. 572-575).

[7]In *Cerebratulus marginatus* the reduced number is sixteen (Coe. '99, p. 441; Kostanecki, '02, p. 272). It should be noted that in some eggs nineteen chromosomes are found instead of eighteen (Pl. 1, Fig. 16). There are three possibilities to account for this irregularity in the number of chromosomes: a,

the history of the chromosomes agrees in the main with what has been described by Griffin in the egg of *Thalassema* ('99).

In passing it might be mentioned that the plasmosomes occur only in the germinal vesicle, chromatic nucleoli alone being present in the other nuclei. Both in the segmentation nucleus and those of the blastomeres the chromosomes arise as fine threads irregularly curved and sometimes attenuated towards the ends.

What is the relation between the plasmosomes and the chromatin masses in the germinal vesicle? As I have not studied the genesis of these elements I am not able to give any definite answer to this question. That each chromatin mass is constantly associated with a plasmosome makes us think that the relation between these cannot be merely a casual one. In all probability the plasmosome gives off some substance to the chromatin masses for the growth of the latter. It is extremely difficult to analyze the cause of the change of the staining capacity of the chromatin masses, because two phenomena take place almost simultaneously; namely, the disappearance of the smaller plasmosomes and the fading of the nuclear membrane. It is, however, probable that the dissolution of the nucleoli is in some way or other connected with the change of the staining reaction of the chromosomes; for the latter phenomenon takes place only in the germinal vesicle, and not at every dissolution of the nuclear membrane in later stages. Although one or two chromatin spherules are found in the larger plasmosome, the relation of the nucleoli and chromosomes is in *C. lacteus* not so close as in the egg of echinoderms, in which all the chromosomes in one period take shelter in the nucleolus. At any rate our case does not

failure of conjugation of a homologous pair of chromosomes at synizesis; b, persistence of idant-free chromosomes (*vide infra*) as late as the metaphase, and c. separation of one chromosome into two. Of these the second may not be the explanation of our case, because of the fact that in some eggs there are thirty-eight chromosomes in a daughter plate at the anaphase of the first cleavage mitosis instead of thirty-six. In all probability our case may be due to the third possibility, although I have no reason to exclude the first. At any rate separation of one chromosome into two or more and fusion of two or more chromosomes into one may have taken place in the course of phylogeny of organisms. Without this assumption we cannot understand how such diversity in number of chromosomes in animals and plants has come about.

seem to support the secretion theory of the nucleolus advocated by Häcker ('99) and recently by Bonnevie ('06), but is in favor of the transportation theory as has been maintained by Rhumbler ('93, p. 351); Lubosch ('02); Hertwig ('02); Hartman ('02); Günther ('03) and others.

III. Centriole and Centrosome.

(*a*) *Centriole.*

In a general way the centriole may be said to be a cell-organ, which undergoes very little change during the complicated processes of karyokinesis. The centrioles show, however, in normal as well as in abnormal cases some variation in size, shape, number and position in the centrosome.

(*a*) The centriole usually lies at the spot to which the astral rays converge, but sometimes it is found eccentrically in the aster. The segmentation centriole seems to drift about in the degenerating aster, since it is situated at no definite place (Pl. III, Figs. 43 and 45). In an enlarged centrosome the position of the centriole is very variable (Pl. III, Figs. 50 and 51).

(*b*) The size of the centriole seems to be fairly constant in all the cells of a given species, although a slight change in its size can be noticed (Boveri's view of proportionality between the size of cell and that of centriole may not be universally applicable, '06, p. 96). In fact at its first appearance, it is a little smaller than in a full-grown aster. A considerable diminution takes place in the young cleavage-centriole found near the conjugating germ-nuclei (Pl. III, Fig. 45). In abnormally treated eggs, *e. g.,* in the $CaCl_2$ eggs, the centrioles vary in size in an extraordinary degree, ranging from the smallest one but a little larger than the vanishing point up to those almost three times as large as the normal size (Yatsu, '05, Fig. 10). Only a few cases have hitherto been recorded, in which the centriole actually enlarges and becomes hollow. Häcker observed in *Sida* the growth of the centriole ('93); Conklin in *Crepidula* ('01, '02), and in *Ciona* and *Cynthia* ('05); Smallwood in *Haminea* ('01, '04); Bonnevie in *Enteroxenos* ('06).

(*c*) The centriole is usually a smooth sphere, but often has a rough surface. Sometimes it elongates into a thin rod, while in other cases a process is sent off from it. In *Asterias* eggs treated with ether, I often meet with V-shaped centrioles. Besides these abnormal cases, rod and V-shaped centrioles occur normally in some forms, *e. g.*, in *Polystomum* (Halkin, '04, pp. 298-299); *Pygaera* (Meves, '02); *Blatta* (Wassilieff, '04); *Zoogonus* (Goldschmidt, '05, p. 619); *Dictyota* (Mottier, '98); *Stypocaulon* (Swingle, '97). The centriole usually divides after amitotic fashion. The Schreiners ('05) have observed in *Myxine* a budding of the rod-like centrioles as has been described by Heidenhain ('96). Mattiesen ('03) seems to have seen that the rod-like centrioles in the egg of a fresh water dendrocœl are divided longitudinally (p. 37).

(*d*) The centriole is usually single or double in the aster of dividing cells and double in the resting tissue-cells and germ-cells before their growing period ("diplosome," Zimmermann, '98). When double, they lie close together, excepting those in enlarged centrosomes (Pl. III, Fig. 52) and in abnormal eggs. Three or four centrioles in an aster have been described in *Paludina* and *Pygaera* (Meves, '02) in *Haminea* (Smallwood, '04, Pl. V, Figs. 25 and 28). Lillie draws six centrioles at the central end of the first maturation spindle of *Chætopterus* egg ('06, p. 208, Fig. 39). The egg of *Cerebratulus* treated with $CaCl_2$ shows an enormous increase in number of the centrioles (cf. Yatsu, '05, Fig. 10).

We may define the centriole as follows: the centriole is a well-defined cell-organ comparable to the chromosome in its inherent power to grow and to multiply by binary fission. It never grows beyond a certain limit (exceptionally enlarges and becomes hollow). It is either single or double, but more than two form abnormal cases (Boveri, '95, p. 60, *et seq.*). We are as yet unable to discern what happens when the centriole passes out of sight during the resting stage and in some centrosomes, just as we do not understand the achromatic state of "chromosomes."

(*b*) *Centrosome.*

The centrosome is very insignificant in the resting asters of *Cere-*

bratulus. Indeed it is even wanting in (*a*) the sperm-, maturation- and segmentation-asters, when just appeared; (*b*) in any asters immediately after division; (*c*) in the asters found in the *pilidium* (Text Fig. A, j and k). In other cases the rays change their nature near the centriole, and give rise to a special zone of archiplasm as is seen in Fig. 58 (Pl. IV). In other cases a homogeneous area is found between the archiplasm and the centriole. This zone is the centroplasm (= centrosome). A remarkable difference can be noticed after different fixing fluids in its sharpness of outline and its affinity for stain. In still other cases the archiplasm layer is lacking, and the rays radiate directly from the centrosome. In the last cases the centroplasm usually contains the central end of the rays (Pl. III, Fig. 56). In large cells the centroplasm, at the division of an aster, enlarges and becomes alveolar, as we shall see later (Pl. III, Figs. 49-53).

In all probability the centriole has the power of transforming either the cytoplasm, or the archiplasm or rays into centroplasm. The ray-system is also beyond doubt produced by the action of the centriole. Some other structures may also be the centre of ray-system as we shall see later, but in what manner is not known. Boveri thinks that the centrosome is the producer of the rays ('00, p. 117). Bütschli ('92) and Rhumbler ('96) tried to explain the ray formation as caused by the hygroscopic nature of the centrosome, and, moreover, according to the latter, the disharmony of the growth of the centrosome and the ray-system is due to a peculiar character of certain colloid substance, such that swelling takes place suddenly when a certain amount of water has been absorbed. But the fact that the rays are formed even when there is no centrosome around the centriole and that the centriole[8] does not change its physical nature throughout the whole process of ray formation makes it difficult to accept the views of the above cytologists. The ray-forming activity of the centriole, however, seems to cease in the latter part of the growth of the aster, as is shown by the eccentric position of the centriole in old asters. It may, therefore, be safely said that

[8]Many cytologists call centriole centrosome.

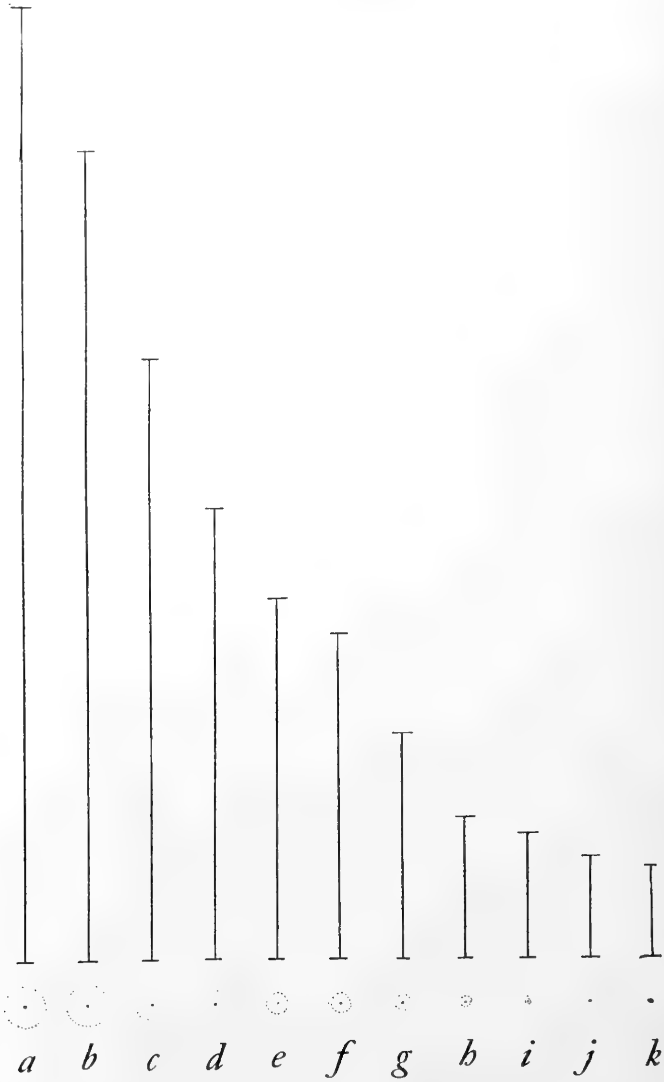


Fig. A. Diagram showing the size relation between the cell and centrosome. The vertical lines represent diameters of cells taken from embryos of various stages (all at the metaphase). × 1666. Below, the outlines of the centrosome of corresponding cells are indicated. × 1666. a—1-cell stage, b—4-cell stage, c—8-cell stage, d—64-cell stage, e—32-cell stage, f—"placula" stage, g—blastula, h—gastrua (a cell of the apical organ), i—young pilidium (an entoderm cell), j—pilidium (an entoderm cell), k—pilidium (an entoderm cell near the mouth). During the process of reproduction the centriole of e has become smaller and that of k larger than those in the original drawing.

the centriole initiates the formation of both the centrosome and ray-system.

In a general way the size of the centrosome is proportional to that of the cell in the same species, as has been pointed out by Boveri ('01, p. 94) and Goldschmidt ('02, pp. 406 and 424). The accompanying diagram (Text Fig. A) shows this relation in *Cerebratulus.* The sections of cells of various sizes (all at the metaphase) were taken at random, their diameters and the size of centrosomes were measured, and were arranged in order. One notices at once the proportional decrease in size of the controsomes as the cells become smaller, while the centrioles remain without perceptible change throughout. Furthermore it should be noted that in the cells of pilidium the centrosome no longer exists. As the centroplasm is present at any place of the cytoplasm (as is seen in the normal fertilization and in artificial production of cytasters) it is clear that the larger the cell the more centroplasm, and consequently *ceteris paribus,* the centrosome can grow much more in larger cells than in smaller ones. But the question why the large cells need large centrosomes will remain unsolved until we come to know the function of the latter.

From what has been said it will be seen that the centrosome is an ephemeral structure produced by the accumulation of centroplasm around the centriole, and "*nur von den Centriolen, nicht aber von den Centrosomen, kann daher gelten, dass sie allgemeine und dauernde Zellorgane sind,*" as has been maintained by Meves ('02, *a* and *b*); Bouin ('04) and the Schreiners ('06, p. 448). Although Boveri hinted at this conclusion as a mere possibility ('01, p. 185), he seems to lay more stress on the centrosome than the centriole.[9] Nevertheless the centrosome theory does not lose its validity for the reason that the centriole physiologically represents what was called centrosome when the theory was first formulated.

In her excellent paper on *Enteroxenos* ('06) Bonnevie advances

[9]Vejdovsky's and Mrázek's ground of denying Boveri's idea of continuity of the centrosome is somewhat different from the above authors, they emphasizing more the *new formation* of a new centrosome in the old, than its *periodical complete* disappearance ('03, p. 555).

a view quite different from what has been said above. The diplosome, found in the tissue-cells and in the germ-cells before their growing period, she identifies as the centrosome.[10] This structure divides bodily into two in spermatogenesis and during the oogonial divisions, while in oocytic divisions "*durch innere Differenzierung*[11] wird das zuerst kompakte Cytocentrum (=diplosome) in eine Hohlkugel umgebildet, und in der Mitte derselben, kommt ganz allmählich ein winziges Körnchen zum Vorschein, das sich während der Metamorphose in zwei Körnchen teilt" (pp. 302-303). These granules she calls centrioles. From this she concludes that the centrosome is a permanent cell-organ and the centriole is not, because it is not present in all the division stages and occurs only in large cells. If one studies her paper critically, it will not be difficult to see that this conclusion rests first upon the fact that in *Enteroxenos* the common type of aster-division is peculiarly modified by the growth of the centriole; and second, upon a misinterpretation of the nature of the diplosome. It cannot be denied that the small granules in her Figs. 7 and 13 (text Fig. D on p. 315) are morphologically as well as physiologically the same as those at the centre of the centrosome of Fig. 1. *The distinction between the centrosome and centriole is, after all, not a question of position, but of morphological structure.* But, on the other hand, it may be argued,

[10] It is interesting to note that Boveri admits that in some cases the diplosome may represent centrioles and not centrosome ('00, p. 201). Meves ('02) and the Shreiners ('06, p. 348) seem to have misinterpreted Boveri.

[11] The centriole must have arisen in the centrosome or division centre during the course of phylogeny. Yet from this it does not necessarily follow that at the present time the centriole is formed from the centrosome. As a matter of fact there exists a naked centriole, and in no cases ever described does the centrosome precede in its formation the centriole. The appearance of the segmentation centrioles after temporary disappearance cannot be called inner differentiation, because they keep their identity during this period. The question naturally arises as to whether or not there is a centrosome destitute of centriole. Excepting the cases in which the absence of the centriole is due to a failure in technique and the centrosome from which the cleavage centriole has gone out of sight, the centrosome without centriole has actually been observed in a few forms at one or both ends of the maturation spindles, such as *Entroxenos*, *Zoogonus*. The disappearance of the centriole in these cases, however, may be due to its precocious degeneration.

that the diplosome is a compound structure, *i. e.,* a centrosome containing a centriole in it (cf. Boveri, '00, p. 93). This argument is not valid, since the same can be said of the ordinary centriole in the centrosome. Who can demonstrate that the centriole in the latter case does not have a thin coating of centroplasm about it?

(*c*) *History of the Centriole in Fertilization and in Cleavage.*

I have demonstrated elsewhere (Yatsu, '04, '05) that centrioles can be artificially produced by a salt solution and verified the conclusion reached by Wilson ('01) that the centrioles can be formed *de novo* in cytoplasm. In other words, after the dissolution of the germinal vesicle the protoplasm has an inherent power of producing centrioles, which is inhibited under normal conditions, and is called forth only by a favorable stimulus.

The results of my study of the fertilization of *C. lacteus* excludes, however, the view that the sperm-centriole is of egg-origin and is produced *de novo* by the action of the spermatozoon (cf. Morgan, '00, p. 506). *The spermatozoon in our case actually brings into the egg the sperm-centriole, which later gives rise to the cleavage centres and those of subsequent divisions.*

1. Sperm Centriole.

(*a*) *Observation.*

A mature spermatozoon is represented in Pl. II, Fig. 20. A slender slightly curved head is followed by a tail a little less than six times as long as the head. In well extracted preparations one observes that the middle piece is a vesicle containing a dark staining axis and a distinct granule near the posterior end (Pl. II, Fig. 21).[12] The subsequent history of the granule shows that it is really a centriole and not a structure produced by concentric extraction, as I ascertained by watching every stage of the differentiation by iron-alum. The granule, it should be noted, resists extraction even until the time the head becomes light blue. In a young spermato-

[12]Retzius ('04) failed to differentiate this granule in the spermatozoon of *Malacobdella grossa* (his Figs. 20-27).

zoon (Pl. II, Fig. 19) the head is composed of two parts; a fine anterior process and a posterior broader part. The middle-piece is much more vesicular than that of the full grown ones. In still younger ones (Pl. II, Figs. 17 and 18) the head is almost spherical and is prolonged anteriorly into a short process. The centriole is present at the posterior end of the protoplasmic covering. After piercing through the egg-membrane the spermatozoon bores into the egg, where a little depression is seen. Then the head shoots into the oöplasm with a whip-like movement of the tail[13] and the latter is quickly drawn in.[14] In many cases the spermatozoon rotates nearly 180°. Very little locomotion takes place within the egg, judging from the fact that all the stages of the formation of the sperm-nucleus,[15] and of the sperm-asters are found near the periphery of the egg.

[13]One may observe a sawing movement of the head, as it gradually pushes in before its final shooting-in. After piercing through the egg membrane, quite often the spermatozoa are found again boring into the membrane from inside instead of into the egg. It should also be noted that spermatozoa are sometimes found boring into the fertilized eggs or even the blastomeres of the two-cell stage. This clearly shows that the spermatozoon has no power of distinguishing the non-fertilized from the fertilized eggs, and that the attraction of the spermatozoon to the egg, at least in the vicinity of the egg, is not due to chemotaxis.

[14]In sections the tail is seen in the egg as described by Kostanecki '02, p. 273). One notices that it has shortened a great deal. Whether a part of the tail is lost at the entrance or is dissolved in the egg, or whether it contracts I have not been able to determine. But it is noteworthy that it increases in its staining capacity after entering the egg (Kostanecki and Wierzejski, '96, p. 336). The tail is sometimes straight (Pl. II, Fig. 27), sometimes it bends on itself (Pl. II, Fig. 26). From this and the fact that in some molluscan eggs the spermatzoon is found coiled up, it may be inferred that the sperm may move in the egg. In fact, by careful watching of the egg of *Cerebratulus*, it may be observed by the disturbance of the yolk granules that the spermatozoon wriggles for a little while before it becomes quiescent.

[15]The sperm head shortens after its entrance into the egg (Pl. II, Fig 24). A construction appears between the head proper and anterior process (Pl. II, Fig. 25). Then the chromatin seems to be drawn into the head proper from the anterior process, which gradually loses its staining capacity. The head proper becomes more and more spherical as the anterior process becomes thinner (Pl. II, Figs. 26-28). Finally the latter disappears (Pl. II, Fig. 29). It may be pointed out that in the egg the sperm-head repeats in reversed order what it did during the later stages of its growth. The curved slender head.

With iron-hæmatoxylin the middle piece of the spermatozoon in the egg stains as deep as the head. In thoroughly extracted preparations, however, the centriole comes into view very distinctly (Pl. II, Fig. 21). A somewhat advanced spermatozoon is represented in Fig. 27 (Pl. II); the middle piece is here rhomboidal. Fig. 28 (Pl. II) shows the same section subjected to further extraction. The centriole has been brought out more clearly into view; the outline of the middle piece has disappeared. It is striking that a fine radiation (non-fibrous rays) converging to the middle piece has been brought to light by extraction. Wilson states that at the formation of the sperm-aster the radial arrangement of alveoles precedes the true rays ('99, p. 13). The same preparation was re-stained with rubin S; the outline of the middle-piece was restored as it had been seen before extraction. The next stage is shown in Figs. 29 and 30 (Pl. II). The vesicular nature of the middle-piece is now more pronounced. The centriole is found at the same place where it previously was. In case the germinal vesicle fails to fade the spermatozoon remains at this stage (Hertwig, O. and R., '87, p. 199; Wilson, '96, p. 149, '00, p. 201; Boveri, '02, p. 44). In eggs in which the germinal vesicle has faded faint rays are next seen around the middle-piece[16] (Pl. II, Fig. 31). Another sperm of about the same stage is shown in Fig. 32 (Pl. II). Here the middle-piece has the outline of a pentagon. Further extracted, a sharply-defined centriole with elongated rays was seen (Pl. II, Fig. 33). The outline of the middle-piece was restored by re-staining with rubin S. The next stage is represented in Fig. 34 (Pl. II). The tail had disappeared and the sperm-rays have increased

therefore, seems to have developed for the purpose of piercing through the egg membrane and of boring into the egg. Later the sperm-head (now sperm nucleus) becomes vacuolated (Pl. II, Fig. 30) and chromatin collects on the walls. Then the chromatin assumes a sphere-like form, which soon breaks up into the chromosomes. As the sperm-nucleus grows, the chromosomes disappear as such, leaving behind a few chromatin nucleoli.

[16] I met with a few cases, in which the sperm-rays have developed before the shortening of the head. Fig. 23 (Pl. II) shows this abnormal case. One notices that the sperm-rays centre in the centriole in the middle piece. Fig. 24 (Pl. II) represents another case, in which rays have precociously formed and the throwing-off of the middle piece vesicle has also taken place.

both in number and in length. Soon afterwards the middle-piece is found thrown off into the cytoplasm, where it eventually fades (Pl. II, Figs. 35 and 36). Now the centriole surrounded by astral rays lies free in the egg close by the sperm-nucleus. How the centriole escapes from the middle-piece I do not know. It may be due to the movement of the sperm-nucleus and aster, the middle-piece being left behind. As a matter of fact I have a few cases in which the middle-piece and the tail together are found detached from the sperm-nucleus at the stage represented in Fig. 29 (Pl. II). (Wilson, '97; Foot and Strobell, '03, their Fig. 9.)

When the centriole escapes from the middle-piece, the centroplasm has not made its appearance, the rays reaching the centriole. Soon after, the central ends of the rays become obscure and the centroplasm is formed. In it the centriole divides into two. The division plane has no definite relation to the egg radius (cf. Pl. II, Figs. 36 and 37). From this stage on two different types may be distinguished in the formation of two daughter asters: (A) in many eggs the centrosome disappears immediately after this. The two naked centrioles are found surrounded by new short rays. As they are separated farther and farther from each other, the rays (fertilization rays) grow to a considerable length (Pl. II, Figs. 37 and 38). A spindle is formed by a secondary connection of the rays between the two asters. (B) In a few cases I find the sperm-aster with much centroplasm (Pl. II, Fig. 39). The rays are coarse and not so numerous as in type A. The centroplasm increases in quantity and eventually becomes spongy (Pl. II, Fig. 40). In it the two daughter centrioles acquire new ray-systems and the old rays gradually fade away. These two types differ from each other simply in the period of aster division owing to different amounts of centroplasm; in type A the aster division is completed very early, while in type B it has not yet finished even as late as a stage shown in Fig. 40 (Pl. II). It should be remarked that type A resembles what was observed by Coe on *C. marginatus,* differing only in one point; that is, in the Neapolitan species a large number of old rays directly pass into the daughter systems ('99, p. 446). Type A resembles the formation of the asters for the second maturation

mitosis described by van der Stricht ('98), Byrnes ('99) and others, while type B conforms in every respect to the mode of aster-division in large blastomeres of our form, which I shall deal with later on.

(*b*) *Literature and General Remarks.*

Despite the fact that numerous studies on spermatogenesis have been carried out, and that the fate of the spermatid-centriole in the formation of the spermatozoon has been followed with great accuracy, yet surprisingly few cases are known, in which the centriole of the spermatozoon has been uninterruptedly traced to the sperm-centriole in the egg during the fertilization processes.

Thanks to the older investigators such as Flemming, Fol, O. Hertwig, it has long been known that the sperm-rays centre towards the middle-piece. At one time it was thought that the middle-piece as a whole was the sperm-centrosome (Doflein, '97, p. 206; R. Hertwig, '98; Boveri, '00). The relation between the middle piece and the centre of the sperm aster was made clearer in the works of Henking, ('90), Fick ('93), Wilson and Mathews ('95), Wilson ('97, '99), van der Stricht ('02, '04). There are, however, but five forms in which the centriole has satisfactorily been traced. Hill ('95) observed the centriole in the middle-piece in *Phallusia mammilata* and traced it to the centre of the sperm aster (Pl. 17, Figs. 13a, 21a-f). His description and figures are, however, insufficient to give a clear idea of his observations. Kostanecki and Wierzejski ('96) saw a centriole in the middle-piece of the spermatozoon in the gonad of *Physa fontanalis,* and in a few cases they found the same granule in the egg with rays around it (pp. 338-339). Erlanger ('97) demonstrated the centriole in the spermatozoon of *Ascaris megalocephala* and traced it to the fertilization stages (pp. 316-320). Boveri ('00) states that one or two granules are found in the middle-piece of the spermatozoon of *Strogylocentrotus lividus* (his Pl. 1, Figs. 14d, e, f, h; Pl. A., Figs. 55 a, b; Pl. 5, Figs. 71, 72). The same granule (centriole) he observes in the centre of the sperm aster (his Pl. 4, Figs. 55b, Pl. 5, Figs. 71, 72). Foot and Strobell ('02, '03) found

that the spermatozoon of *Allolobophora foetida* contains two centrioles at each end of the middle piece, and the posterior one persists as the sperm centriole ('03, p. 366). In *Cerebratulus lacteus,* as we have already seen, it is rather easy to trace uninterruptedly the centriole from the young spermatozoon to that of the sperm aster, owing to the fairly large size of the centriole and the vesicular nature of the middle-piece (Yatsu, '07).

As to the fate of the middle piece in the egg. In most forms, I think, the middle-piece fades *in situ* as soon as it enters the egg, thus leaving the centriole free in the oöplasm. The rapid degeneration of the middle-piece makes it in most cases almost impossible to follow its history in the egg. The throwing-off of the middle-piece or, in other words, the escape of the centriole from the middle-piece, seems to me a very rare phenomenon. Besides *C. lacteus* this has been observed in only one form.[17] In *Toxopneustes variegatus* Wilson, ('97, '99, '00, p. 188, Fig. 12) observed that the middle-piece becomes detached from the nucleus and is cast to one side, as a dark staining granule that degenerates *in situ.* The rays focus at the basal point of the nucleus, where the centriole appears ('99, p. 14). Whether the centriole lies in the middle-piece or between it and the nucleus he was not able to determine ('97, p. 371).

The question naturally arises as to whether the centriole or the centriole and centrosome together are brought into the egg by the spermatozoon.[18] But in *Cerebratulus lacteus* the condition is quite different. The granule found in the middle-piece is a little larger than the ordinary centriole, it is true, but just after its escape from the middle-piece it has no centrosome at all. Even if the centrosome be present in the middle-piece, it must degenerate in the oöplasm. In our case at least, it may safely be concluded that *the centrosome in the sperm aster is derived from the egg substance.*

[17]Field ('95) states that in *Asterias* the separation of the middle piece (mitosome) near the place of entrance into the egg as was observed by Pictet and Cuénot (p. 225). But judging from his statement that the spermatozoon devoid of the mitosome is capable of fertilization, the nature of this body can be questioned.

[18]Boveri's figures represent spermatozoa already rotated. The sperm-head with naked centrioles might be found before its rotation.

2. Cleavage Centriole.

After the formation of the egg nucleus, the egg centriole lingers for a little while in a niche of the nucleus, but it soon disappears, (Pl. IV, Fig. 68). The sperm nucleus, with two asters connected by a spindle comes in contact with the egg nucleus not far from the place where the latter was formed. The asters sometimes precede (Pl. III, Fig. 41), sometimes follow the sperm nucleus (Pl. III, Fig. 42) (cf. Coe, '99, p. 447, Kostanecki, '06, p. 17). At the time of the conjugation of the germ-nuclei or a little later, the sperm asters become irregular, the rays curving like a fountain (Pl. IV, Fig. 68). Singularly enough no considerable increase of the centroplasm takes place. The centriole may take any place in the degenerating centrosome—in many cases away from the centre of the rays; sometimes close to the nuclear walls, sometimes quite far from it. This fact suggests strongly that there may have been a current in the cytoplasm at the conjugation of the germ-nuclei.

In order to decide the origin of the cleavage centrioles, I have studied serial sections of twenty-three eggs at the stage when previous observers lost the centrioles in the egg of *C. marginatus* (Coe, '99, Kostanecki, '02). The result was that six eggs show two centrioles, fourteen eggs one centriole and three eggs none. Figs. 43 and 44 show the sections of the egg in which two centrioles are found at the critical period. Are the two centrioles new formations or the same ones as those in the sperm asters? It is extremely difficult to decide this question owing to the fact that one cannot follow the history of the centrioles in the living eggs. But it is clear that these centrioles are not on their way to degeneration, but they have recently acquired new activity since they have a small ray system around them. Prior to the conjugation of the germ nuclei, the centrioles are invariably present (Pl. III, Figs. 41 and 42), and in some cases the centrioles have come to possess a new ray system even before the coming together of the germ nuclei (Pl. III, Fig. 45) (Coe, '99, pp. 451 and 457). From this it may be concluded that *in some cases the sperm centrioles survive through the critical period, while in many they disappear during this stage.* It should here be noted that the centrioles dwindle considerably in size just before the conjugation

of the germ nuclei, and that at this particular period the centroplasm is fixed always very poorly. Any one who has had experience in fixing has surely noticed that the same treatment acts differently upon different lots of eggs according to their "physiological state" so to speak. (Mrs. Andrews, '97, pp. 16, 31.) Even in one and the same lot one finds eggs excellently fixed side by side with poor ones. Especially at the moulting stage the centriole is extremely sensitive towards fixing fluids. Wilson ('01a) expresses the difficulty in fixing the centrioles at the division stage of the aster. Vejdovsky and Mrázek ('03) state that they could not follow the division of the sperm centriole owing, I imagine, to the impossibility of getting satisfactory fixation at his particular stage (pp. 502-503). Taking into account the above difficulty in fixation it is certain that the sperm centrioles pass through a stage when they are liable to be destroyed by fixing fluids. Only such centrioles as happen to regain their activity at an earlier period, and come to be less susceptible to the fixing fluid, can escape from being dissolved (cf. Wilson, '00, p. 214). Despite the temporary disappearance of the sperm centriole, I conclude, therefore, that *the cleavage centrioles are identical with the sperm centrioles* (Kostanecki, '06, p. 60).

(*d*) *Division of the Centrosome.*

1. *Observations.*

For the study of the moulting of the centrosome, no stages can be better than the formation of the second cleavage centrosomes. This process begins in *C. lacteus* at a late prophase of the first cleavage mitosis. The centriole becomes double at the end of the spindle. The centrosome at this stage has not increased in size (Pl. III, Fig. 47). It should here be noted that the division of the centriole and the increase of the centrosome are two independent phenomena. The centriole divides irrespective of the spindle axis, *e. g.*, perpendicular in Fig. 47 (Pl. III), and obliquely in Fig. 50 (Pl. III) (cf. Boveri, '00, p. 43). Neither does the division plane coincide with the short axis of the centrosome (Pl. III, Fig. 50). In the meanwhile en-

largement of the centrosome takes place. The direction of its growth is not determined by the centrioles, but by the general organization of the egg (cf. Mark, '81, p. 526, Boveri, '00, pp. 48, 107).[19]

Then the centrioles move apart from each other in the centrosome (which differs from Coe's observation, '99, p. 459). The separation seems in some way correlated with the growth of the centrosome. As the centrioles move apart two dark staining fibres can be seen between them (Pl. III, Fig. 48), but no central spindle (netrum) is present (Schreiners, '06, p. 331, Fig. 179). One of the connecting fibres may remain a little longer than the other (Pl. III, Fig. 51). The centrioles then take their definitive position, the line connecting two centrioles being perpendicular both to the vertical egg axis and to the spindle. Their position is, as already mentioned, governed by the shape of the centrosome, which is in turn dependent on the general organization of the egg. The centrosome is alveolar in structure (Vejdovsky, '88, p. 19, Wilson, '99) and has the form of a sausage flattened horizontally. It now grows very rapidly, partly at the expense of the chromosomal fibres, partly by modification of the archiplasm. The chromosomal fibres shorten without thickening, which suggests the hauling-in of a rope as Rhumbler states in the case of the polar rays ('96, p. 607), the fibres being gradually metamorphosed into the centroplasm (Wilson, '95, p. 2, '96, p. 77, '01, p. 387; Coe, '99, p. 459). The chromosomal fibres completely disappear, and the chromosomal vesicles are found actually in the centroplasm. The centrosome is now a flat sheet and the centrioles have moved to a point near the outer periphery of the centrosome. Here they acquire a ray system but are at first devoid of the centroplasm. Then the old centrosome begins to disintegrate. Hand in hand with this dissolution the rays around the centrioles become more and more distinct; often a spindle may be formed as the result of secondary connection of the rays. As the

[19]In this connection it is interesting to mention that in the egg of *Arbacia* centrifugalized and afterward fertilized, the second cleavage takes place always horizontally, as I was told by Professor Morgan. I think in the eggs thus treated a vertical movement of the egg material (which tends to restore the original structure) controls the definite shape of the first cleavage centrosome and this in turn the position of the centrioles for the next cleavage.

aster enlarges the centrosome comes into view for the first time, and the mother rays gradually fade away as the daughter rays grow stronger. When the old rays have entirely disappeared, the moulting of the aster is completed.

From the above observation on the formation of asters for the second cleavage of *C. lacteus* it will be seen that:

(*a*) the centrosome does not divide; it being formed separately from the beginning.

(*b*) in no stages does a reduction of the centrosome take place.

(*c*) the centriole retains its identity throughout the whole process; neither disappearance nor enlargement of the centriole takes place.

2. *Types of Centrosome Division.*

Three different views have been held regarding the formation of daughter centrosomes from a preceding one, if we except the direct division of the centrosome, which takes place in some small cells, *e. g.*, the spermatocytes and cleavage cells of *Ascaris.* As we shall see later, none of the views expresses the general mode of the division, but there are actually three different types (Meves, '99, p. 499 *et seq.*).

A. Formation of daughter centrosomes by reduction (Boveri, '00). It is quite universal, Boveri believes, that the centrosome first enlarges (Text Fig. B. II *cf.* his Text Figs. pp. 102-103), and in it the centriole divides (this may happen long before). Suddenly the centrosome differentiates into two parts, a central "active" core and an outer disintegrating part. This process he calls a reduction of the centrosome ('00, pp. 97, 101). The reduced centrosome now divides into two. Here the division of centrosome actually takes place. The term "reduction" is, I think, not well chosen, since it does not express what really happens. The grown centrosome does not all of a sudden separate into two parts, as Boveri thinks, but thicker centroplasm flows towards, and collects itself around, the centriole, and thus slowly the centrosome of the next generation is formed within the old. Example, formation of the second cleavage centrosomes in *Cerebratulus marginatus* (Coe,

'99), in *Rhynchelmis*[20] (Vejdovsky and Mrázek, '03); that of *Thalassema* (Griffin, '96, '99) may belong to this type (*vide infra*).

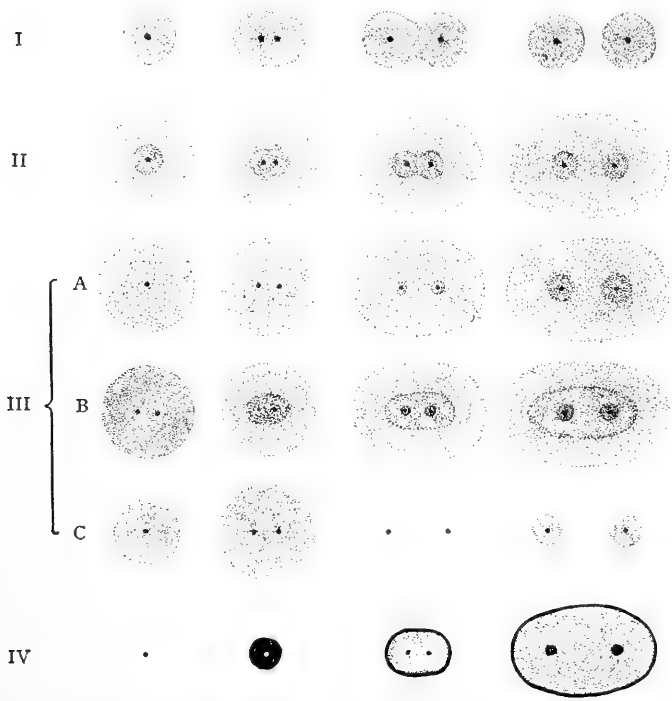


Fig. B. Diagram showing various types of the formation of daughter centrosomes from a single original one.

B. Formation of daughter centrosome by moulting (Vejdovsky, '88, Vejdovsky and Mrázek, '03). Disagreeing with Boveri's view of reduction of the centrosome, Vejdovsky and Mrázek maintain that a daughter centrosome arises within the old one, and a grand-daughter centrosome within the daughter and so on, like a series of ring waves as aptly expressed by His ('98, p. 442-443). They deny Boveri's conclusion on the ground that the daughter centrosome is a

[20]In this form the centrioles acquire new ray-systems before the division of the centrosome.

new formation and not due to reduction (p. 529, etc.). But it is certain that the material for the new centrosome must come from the old, as already mentioned. This endogenous formation of daughter centrosome may well be called "moulting," in lieu of Boveri's reduction. So much for the controversy in interpretation. Vejdovsky and Mrázek give another type besides that described under A. The centrosome enlarges and the centriole which had been divided into two acquires a ray system and a centrosome (Text Fig. B). The old centrosome degenerates. In this type the centrosome never divides, but is formed separate from the beginning. Example: formation of the second cleavage centrosomes in *Glossiphonia* (=*Clepsine*) (Vejdovsky and Mrázek, '03, in *Cerebratulus lactus,* in *Serpula* (Soulier, '06, pp. 458-459). A critical study of the literature will convince one that this type of centrosome formation is the most prevalent of all; in fact all the known cases, except those mentioned under A and C (*vide infra*) may, I think, be included under this type. Of course in very few cases is the process carried out with diagrammatic clearness as in *Cerebratulus.* In most cases it is modified by various irregularities. Of these three may be mentioned.

(*a*) Owing to the precocious fading of the mother centrosome the centriole becomes naked and divides into two. Around these the daughter centrosomes are formed. Example, in the spermatocytic division of *Geophilus* (Bouin, '04).

(*b*) The mother centrosome disappears after the centriole has divided. Two naked centrioles exist for a time, as shown in Text Fig. C. Example, the formation of the second maturation centrosomes in *Thysanozoon* (van der Stricht, '98) and in *Limax* (Byrnes, '99).

(*c*) The centriole divides and before the separation of the daughter centriole a common centrosome is formed around them. After it has enlargel two grand-daughter centrosomes appear within it (Text Fig. III B.). Erlanger's account ('98) of the egg of *Sphaerechinus* may conform to this process. It is doubtful whether or not Griffin's case of *Thalassema* represents type A, for the reason that he actually figures an enlargement of centrosome. Might it not be the case that the daughter centrosome disappears in the mother centro-

some before the grand-daughter centrosome appears? (Cf. Griffin, '96, Fig. 13.)

(*c*) Formation of daughter centrosome by the enlargement of the centriole (Wilson, '95, Conklin, '04, Bonnevie, '06) (Text Fig. B. IV). The centriole enlarges in the centrosome and the two new centrioles are formed *de novo* within it. The centrosome is, therefore, a transformed centriole. No division of centrosome takes place. This view is characterized by the fact that at the division of the aster the cell passes a stage in which no centriole is present. Wilson expresses a similar view in the case of *Toxopneustes* ('95, p. 463, '01, p. 584). Boveri suggests this mode as a mere possibility at the formation of the second cleavage aster of *Ascaris* ('01, pp. 78 and 98). Example, *Crepidula, Cynthia,* and *Ciona* (Conklin, '01, '04, '05), in *Enteroxenos* (Bonnevie, '06).

Recapitulating the types:

(1) Direct division.—The centrosome is bodily divided into two daughter centrosomes (centrosome divides).

(2) Moulting.

(*a*) Division.—A new centrosome formed around the centrioles within the old one is bodily divided into two centrosomes (centrosome divides).

(*b*) Separate formation.—The centriole divides in the mother centrosome and around the products the daughter centrosomes appear separately (centrosome does not divide).

(3) Enlargement of the centriole.—The centriole grows and becomes the centrosome. Two new centrioles appear in it, and enlarge into centrosomes (centrosome does not divide).

3. *Cycle of Centrosome.*

As a summary of this section the cycle of the centrosome in *Cerebratulus lacteus* from the sperm-centre as far as the second cleavage center will be given. The centrosome I, *i. e.,* that of the first generation, makes its appearance soon after the throwing-off of the middle piece vesicle. The centriole then divides into two. Centrosome I fades away, and new ray systems are formed around the two

centrioles. By this time the germ-nuclei fuse. Centrosome III (that is, the first cleavage centre) appears in the now degenerating centrosome II.[21] Centrosome III enlarges a great deal and in it centrosome IV is formed. This becomes the centre of the second cleavage mitosis. This cycle agrees with Vejdovsky and Mrázek's observation on the egg of *Glossiphonia* (*Clepsine*). That *the fourth centrosome becomes the centre of the second cleavage,* is, I think, an almost universal phenomenon during the early development of animal eggs.

IV. Rays and Spindle.

(*a*) *Terminology.*

Throughout the present paper, I shall use the terms in the following sense:

Pole rays—the entire group of rays radiating from the astral center (Rhumbler).

Polar rays—rays found in the region of the "cône antipode" (Rhumbler).

Intermediate rays—rays found between the polar and equatorial rays.

Equatorial rays—rays near the division plane of the cell (Rhumbler).

Sheath rays—spindle-like sheath surrounding the spindle formed by the fusion of the equatorial and a part of the intermediate rays.

Sheaf rays—rays laid down parallel to the spindle in the future cleavage plane ("gerbe de séparation," Bouin).

Spindle = central spindle; "central" is dropped, because it is sometimes found outside the chromosomal fibers, *e. g., Rhynchelmis* (Vejdovsky and Mrázek, '03, Fig. 46).

Chromosomal fibers = "Zugfasern" = Mantle fibers.

[21]Kostanecki compares the formation of centrosome III after the "pause" with the reappearance of rays after the treatments of cooling, etherization, etc. ('06, p. 65). It might be worth pointing out that these two phenomena are of entirely different nature; in the former a newer aster appears within the old, while in the latter the very same aster becomes visible.

Interzonal fibers—fibers found between the separating chromosomes at the anaphase and telophase. These should not be confounded with the spindle fibres exposed between two daughter chromosomal plates.[22]

(*b*) *Observations.*

Many views have hitherto been expressed as to the nature, formation and function of the rays and spindle, yet, when they are critically examined, what we actually know at present is surprisingly little. Any attempt to formulate such a highly intricate mechanism based on the body of evidence we have at hand seems premature. In the present section, therefore, I shall not go into the general discussion, but I shall confine myself to the description of a few observations, which have a direct bearing on this subject.

As has been maintained by some cytologists, the rays, I think, are physically modified hyaloplasm of fluid consistency (Mark, '81, p. 528; Wilson, '01a, pp. 544, 549; '01b, p. 385). The effect of ether, cooling, etc., is to bring the rays quickly to the original hyaloplasmic state. Evanescent as they may seem, the rays are, under normal conditions, fairly persistent structures, as shown by the fact that at the moulting of the centrosome the old rays linger for a long while even after the central ends of the rays disappear. It is extremely difficult, therefore, to conceive that the rays represent rapid constant currents of hyaloplasm as Teichmann ('03) and Bonnevie ('06) maintain (Rhumbler, '96, p. 583). It might be mentioned in this connection that the pole rays of the first maturation mitosis remain unchanged for three or four hours unless fertilized.

In fixed material the rays may be divided into two classes according to their nature: (a) fibrous, and (b) non-fibrous (Fol, '91,. The former are actual fibers imbedded in hyaloplasm with microsomes attached to the surface. Tracing the fibrous rays peripherally, one always finds straightened alveolar walls forming non-fibrous rays or, as sometimes called, "Dotterstrahlen."[23] In Fig. 54 (Pl. III)

[22]All the fibres seen between the two daughter chromosomal plates, including both the spindle and interzonal fibres, were called by Mark "interzonal filaments" ('81, p. 198).

[23]The "Dotterstrahlen" of older writers simply mean rays, since the cytoplasm was called "Dotter" in some cases.

the fibrous ray stops at X, while the non-fibrous ray reaches the periphery. The formation of any aster in the alveolar plasm shows that non-fibrous rays usually precede fibrous ones. I have already mentioned a case in which the rays (non-fibrous) of a very young sperm aster were brought to view only after thorough extraction (Pl. II, Figs. 27, 28 and 32, 33) (cf. Wilson, '99, p. 14).

Rays are formed not only under the influence of the centrioles but also under that of many other structures. In the blastomeres of a teleost, *Coregonus albus,* for instance, a special group of rays is formed along the inner side of the karyomere groups (Pl. III, Fig. 56). A section of an egg of *Asterias* accidentally crushed when alive shows distinct rays along the flow of alveoles (cf. Ziegler, '04, p. 556). Parasite asters on the rays have been seen in the egg of *Asterias* (Pl. III, Fig. 55).

The distribution of the pole rays seems to be influenced by that of the hyaloplasm in the cytoplasm as is seen in the fan figure ("Fächerkern"). In etherized eggs of *Asterias,* one often observes funnel-shaped asters.

In many cases the rays are formed in homogeneous plasm[24] (quite different from Bütschli-Rhumbler's explanation). In Fig. 56 (Pl. III) the central ends of the rays are in a homogeneous hyaloplasmic area. Fig. 58 (Pl. IV) is the central aster of the first maturation figure in the nuclear area (residual mass of the germinal vesicle). It is noteworthy that the sperm aster can enter the nuclear area without being distorted (Pl. IV, Fig. 59). Quite often one finds the sperm aster half in.

Rays have a peculiar tendency to elongate toward any formed body, such as chromosomes, or degenerating nucleoli. Fig. 60 (Pl. IV) shows a maturation aster. Three groups of rays are here, as it were, fishing chromosomes. In the middle one it should be noted that a ray is bent so as to meet the chromosome. Apparent splitting of rays may be due to the above characteristic as in the case of Fig. 61 (Pl. IV), where one of the asters has precociously divided.

[24] This is more likely intra-microscopically or potentially alveolar (Veidovsky, '88; Wilson, '99), yet none the less it appears homogenous in the living and granular in the fixed state. Rhumbler calls this "Protoplasma ohne erkennbare Waben" ('96, pp. 544, 545).

Whatever the function of rays may be, they are the expression of an attraction toward the centre. Fig. 62 (Pl. IV) shows a case in which the rays of the sperm aster have pulled the chromosomes of the maturation mitosis toward the centres (cf. Henneguy, '91, p. 417, Fig. 17, or, '96, p. 350).

The spindle fibres are made up of hyaloplasm more highly modified than that of pole rays. The spindle may be removed as a whole by the currents and retains its entity even after the egg is crushed (Ziegler, '95, p. 385; Mathews, '07, p. 90). In the degenerating eggs of *Cerebratulus* (kept unfertilized for five or six hours) the spindle is found without perceptible change, while the pole rays fade always earlier. It is interesting to note that the rays of two asters, when they come near, have a tendency to take the form of a spindle, as in the case of the sheath rays. Figs. 63-65 (Pl. IV) show this relation very well. In the last figure a well developed spindle is seen between the two asters. Fig. 66 (Pl. IV) shows a spindle between the sperm aster and maturation aster; Fig. 67 (Pl. IV) represents that between two sperm asters.

Crossing of the rays takes place not only between two asters connected by a spindle but also between two separate asters. In *Cerebratulus* the rays of the degenerating egg aster and those of the sperm aster do not form a spindle, but invariably cross one another, an accumulation of granular precipitation being present between the asters (Pl. IV, Fig. 69). In this case the crossing may be interpreted as due to a non-simultaneous action of two asters and local disturbance (Rhumbler, '98, p. 547, '03, pp. 520-522). But this explanation is far from satisfactory when one tries to apply it to the ordinary case of the crossing at the metaphase, which is a constant process and not an accidental one (Meves, '99, p. 524).

The fountain figure is found in the egg of *Cerebratulus* in three places: (a) around the cleavage plane when the constriction is nearly completed (Text Fig. C. 5 on p. 386); (b) in the sperm rays immediately prior to the conjugation of the germ nuclei (Pl. IV, Fig. 68), and (c) in the polar rays of the blastomere cleavages at a late anaphase and the teleophase ("Polfontain" Rhumbler) (Text Fig. C. 3 and 4 on p. 386). The anti-spindle figure described by

Coe in the egg of *C. marginatus* at the anaphase of the first cleavage mitosis ('99, Fig. 31) is seldom met with in that of *C. lacteus.* It, however, occurs so seldom that it would be more natural to look upon this figure as the result of fixation or some other abnormal conditions. Spiral asters are also seen at the poles of the maturation spindle, but their occurrence is so inconstant that the figure should be interpreted as due to some accidental disturbance rather than to any constant cause.

V. Cytodieresis.

(*a*) *Observations.*

In the hope of finding some key to the solution of the mechanism of cell division, the first and second cleavages of the egg of *C. lacteus* have been studied. For the sake of convenience the results will be described under five headings:

1. *Movement of Chromosomes and Centres.*

In order to determine the relative movement of the chromosomes and the centres, I have measured sections of twenty-five eggs at the metaphase and at the mid-anaphase.[25]

Metaphase—46.73 microns from the equatorial plate to the periphery (through the center).
16.415 microns from the equatorial plate to the centriole.

Midanaphase—46.90 microns from the middle plane of the spindle to the periphery (through the center).
19.09 microns from the middle plane of the spindle to the centriole.
10.78 microns from the middle point of the chromosomes to the centriole.

The movement of the chromosomes is 8.310 microns.

The movement of the centriole is 2.675 microns.

[25]Only vertical sections were made use of in which the plane passed the two opposite centrioles.

Difference is 5.635 microns.

The elongation of the egg is 0.13 microns.

From this it will be seen that the chromosomes move much faster at this stage than the centrioles (cf. Ziegler, '95, p. 383). The lateral stretching of the karyokinetic axis takes place later on.

2. *Spindle.*

At an early anaphase the spindle is convex in outline at the middle. Approaching the telophase, however, the fibres straighten themselves, the width of the spindle being consequently reduced to one half or sometimes still less (Text Fig. C. 1-4). After the daughter nuclei are formed, the spindle fibres are bent toward the vegetative pole (Text Fig. C, 5). This bending is not due to the cell constriction, because this takes place long before the cleavage furrow reaches the spindle, and moreover against the pushing-in of the vegetative furrow. In later stages, of course, the animal furrow accentuates the bending. It may be pointed out, therefore, that this common phenomenon of spindle bending is an expression of protoplasmic movement.[26]

3. *Centrosome.*

At the anaphase the centrosome is sausage-shape, placed horizontally perpendicular to the spindle. At a late anaphase it flattens and spreads out at the expense of the chromosomal fibres (Text Fig. C. 4—horizontal section). The nature of the centroplasm changes from homogeneous to alveolar and now it does not appear so viscid as it has been. The centrosome is gradually bent downward so that the centrioles are no longer found on the same horizontal plane as the spindle, but far below the latter (Text Fig. C 3). As I have already mentioned, the spindle fibers by this time are curved toward the vegetative pole and the karyokinetic axis, therefore, takes the form of an inverted W (Text Fig. C. 5). Later the nucleus too yields to the shape of this curve. The centrioles go to the extreme end of the centrosome, where they acquire new ray systems. Often

[26] It is noteworthy that in the egg of *Pedicellina americana* the spindle of the first cleavage is bent towards the animal pole, as I was informed by Dr. Dublin.

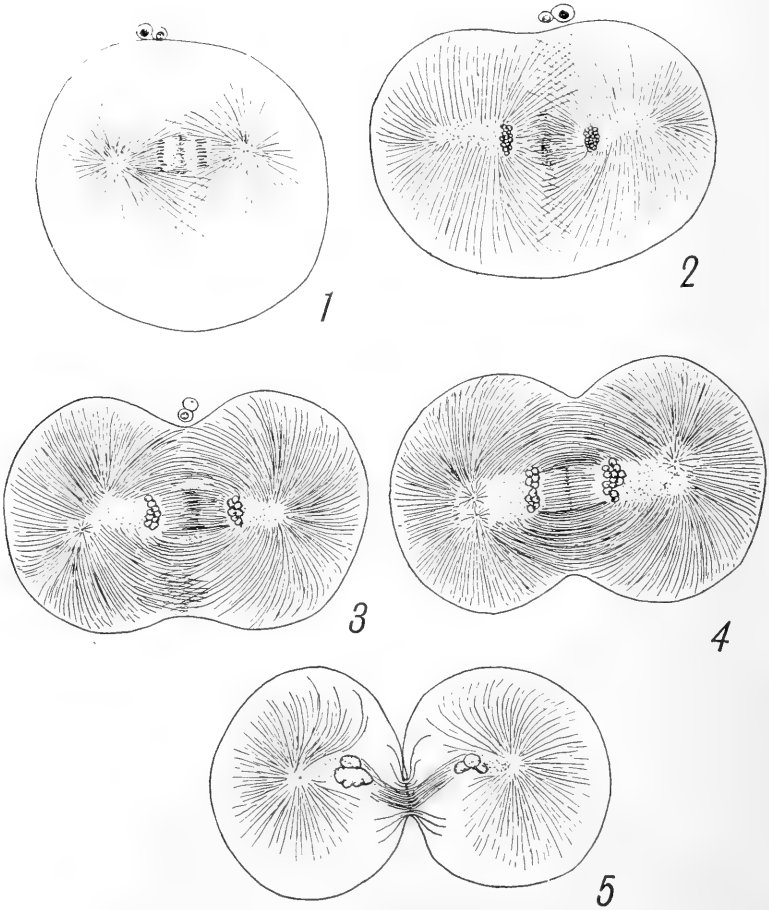


FIG. C. Five stages of the first cleavage, $\times$ 400. 1. Anaphase of the first cleavage mitosis. Crossing of rays, and the position of the spindle. Difference in distance between two centrioles at either end; 2. Telophase of the first cleavage mitosis. The centrosome has enlarged and been bent downwards. The crossing of rays has begun to loosen. A faint indication of fountain figure is seen at the pole regions; 3. Telophase, a vertical section through the poles and a centriole. Constriction has begun on both the animal and vegetative sides. Sheath spindle has been formed. Fountain figures at the poles of the spindle have become more distinct; 4. Telophase, a horizontal section through three centrioles (about the same stage as 3). Sheath spindle is very well formed. Centrosome has greatly enlarged. The distance between the rows of karyomeres is approximately the same as the original length of the spindle; 5. Late telophase (a vertical section). Sheaf rays have been formed. Fountain figure in the equatorial rays.

the new asters are formed outside the centrosomes. The centrosome in section gives a granular appearance as it degenerates, and remains for some time between the new aster and the nucleus. A similar change takes place during the second cleavage.

4. *Rays.*

From the metaphase to the mid-anaphase the rays are straight and comparatively short; they are longer toward the vegetative pole than toward the animal pole (Text Fig. C. 1). Consequently, the rays cross one another much more in the vegetative region. At this stage careful focussing shows that the non-fibrous rays reach the periphery. Through the transformation of these non-fibrous rays into fibrous a beautiful display of rays ensues. Some of the rays abut against the surface of the egg. Soon afterward the elongation of the centrosome takes place. The rays are no longer straight; the polar rays assume a fountain figure. The curvature of these rays is more and more marked in later stages. Now the rays at the "crossing" have a tendency to dissociate or draw themselves apart. Text Fig. C. 4 shows the next stage where the climax of the ray formation has been reached. One striking feature of this stage is the formation of the sheath rays around the spindle, due to the fusion of the equatorial rays and a part of the intermediate rays. These spindle-shaped sheath rays seem to occur in a good many forms (Wilson, 01b, p. 383, his Figs. 51-57). It should here be noted that the cleavage furrow, as it deepens, cuts apart the sheath rays in the middle. It is remarkable that these rays are cut without being bent inwards, and still more so, since the rays thus separated begin to turn away from the cleavage plane, taking a fountain figure (Text Fig. C. 5). This should not be confounded, as I have already mentioned, with the antispindle figure of the anaphase.

5. *Cell Constriction and Mid-body.*

About an hour after fertilization,[27] soon after the formation of the second polocyte, a furrow appears along the animal hemisphere.

[27]In the eggs taken from individuals, which had been kept for two or three days in an aquarium, the maturation processes go on very slowly, and the cleavage furrows appear an hour and a half after fertilization.

Immediately afterward the vegetative furrow cuts in at a rate two or three times slower than that of the animal one. Sometimes the vegetative furrow is very much reduced, and the constriction is accomplished almost entirely by the animal furrow.[28]

In sections of the egg of a late prophase we see precipitated nuclear fluid around the spindle. At the metaphase this fluid takes its definitive position around the equator of the spindle ("Bütschli's space," Rhumbler) (Text Fig. C. 1-3). As the chromosomes move apart toward the poles, the nuclear fluid is taken into the spindle in the form of dark granules. The spindle fibres too seem to absorb the fluid into themselves, as shown by the fact that they are thicker and stain darker in the middle. Spread over the animal half of the egg is found a thin layer of cytoplasm rich in hyaloplasm. This layer is thickest in the middle and gradually thins out toward the equator of the egg. This hyaloplasmic layer sends off a vertical layer to meet the nuclear stuff of Bütschli's space (Text Fig. D 1 and 2). At later stages this hyaloplasmic cytoplasm is also found over the vegetative pole. Thus the future cleavage plane is foreshadowed with this plasm ("Diastem" His). Now this vertical septum begins to become less dense. The cleavage furrows cut in along this thin hyalo-plasmic cytoplasm. As the constriction proceeds a new ray system is formed parallel to the spindle (sheaf rays) (Text Fig. C. 5). Dark staining granules are laid down at the middle of both the sheaf rays and spindle fibres. Later both kinds of fibres are bundled into a sheaf. The chromatic granules fuse together and form a ring (mid-body).

(*b*) *General Remarks.*

The methods which have hitherto been employed for the study of cell-division mechanism may be classified under three categories, namely, (a) observations on the normal processes of cell division either in the living state or in fixed material, (b) the study of cell division under modified conditions, (c) imitations of phenomena

[28] This mode of cleavage takes place, as I have often noticed, in artificial parthenogenesis, since the egg nucleus usually lies near the animal pole (cf. Morgan, '99, p. 452).

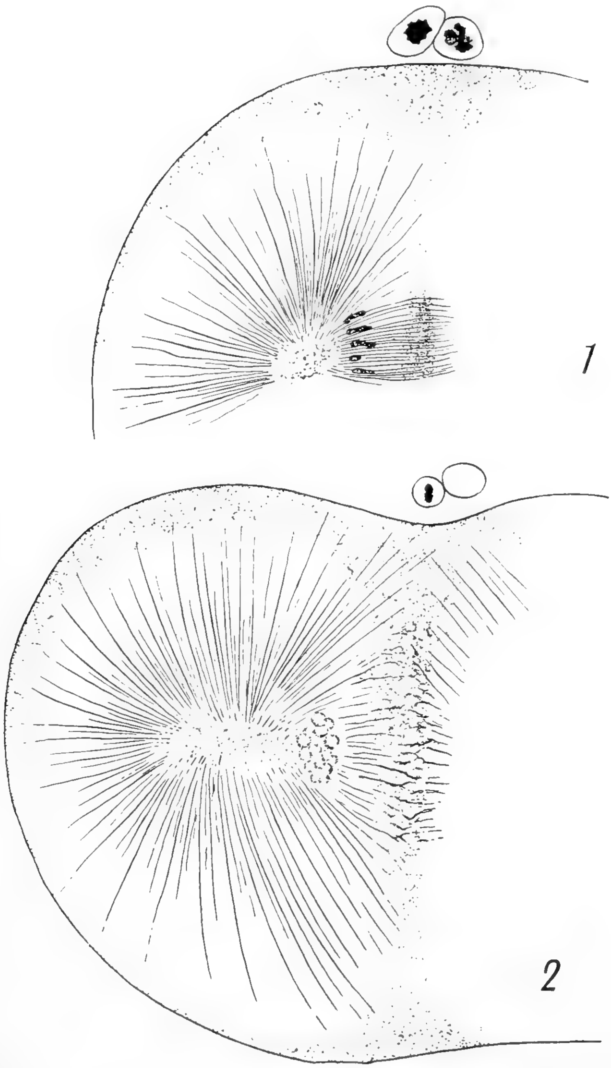


FIG. D. Portion of vertical sections through the egg at a late anaphase and at the telophase respectively, showing the peripheral plasm layer rich in hyaloplasm and vertical plasm layer mainly composed of nuclear fluid. × 866.

of cell division by means of inorganic objects. The last method is a rather dangerous one. Only with the greatest caution should one apply the simulacra thus obtained to our problem. Another line of attack may be offered as a fourth method. This comprises cutting and compressing experiments performed on living cells at different periods of division. How important this method is, may be illustrated by Wilson's experiment on the egg of *Dentalium*. The formation of the polar lobe is naturally interpreted as due to the action of the two centres in the egg (cf. Bonnevie, '03, p. 101). Wilson ('04) found out by a simple cutting experiment that the polar lobe is formed in enucleated egg fragments free from asters (Carazzi, '05, p. 15). Simple as it is, by this method one can test the validity of interpretations hitherto proposed. Although this has been singularly neglected, yet I think the mechanism of cell division will in future be studied most advantageously along this line.

What actually happens during cell division is the rounding-up of the cytoplasm around the two centres (Rhumbler, '97, p. 705, Morgan, '99, p. 521, Teichmann, '03, p. 316). This is undoubtedly the resultant of several factors. Of these the following may be mentioned: The surface tension is usually disturbed at the farthest point from the centre, causing pseudopodia or irregular outlines (Erlanger, '97c, p. 344, Rhumbler, '01, p. 63, 65 and 69, Conklin, '02, p. 94, Boveri, '03, pp. 3 and 5, Jolly, '04, pp. 504 and 505, *e. g.*, Fig. 11 70h 38, Fig. 10 3h 40, 7h 48). As Boveri thinks, this phenomenon seems to be very important for the explanation of the formation of the constriction. By this factor alone the egg should divide by the vegetative furrow. But in reality the animal furrow is the first to appear and in some cases the vegetative furrow is very insignificant. This, as often has been pointed out, is due to the position of the nucleus. The nuclear fluid after the dissolution of its walls takes the form of Bütschli's space (Bonnevie, '06, Körnerhülle, p. 286.)[29]

[29]Conklin thinks that the nuclear fluid escapes from the poles at the fading of the nuclear membrane. This is true for the formations of the rays which give rise to the spindle. But it cannot be doubted that the greater part of the nuclear fluid remains *in situ* and later forms Bütschli's space as Rhumbler maintains.

The nuclear stuff thus exuded, instead of forming the membrane as Rhumbler believes, plays a part in reducing the density of the cytoplasm along the future cleavage plane. The formation of the diastem cannot entirely be attributed to the nuclear fluid, since in some cases the quantity of the fluid is too small to modify the whole cleavage plane. There must be some other factor to produce the diastem. Whatever the cause may be, the diastem is of very common occurrence and any explanation of the mechanism of cell division will not be perfect, unless that solves the problem of its formation (Henneguy, '91, p. 408, Bambeke, '96, p. 34, His, '98, p. 14, Conklin, '02, p. 95, Rhumbler, '03, p. 513, Gurwitsch, '04, p. 326). At the same time in certain kinds of cells it cannot be doubted that cytoplasmic currents play an important rôle in division processes (Conklin, '99, '02, Bütschli, '00).

Appendix: The explanations of cytodieresis hitherto proposed may be classified under five categories; viz.

(*a*) Explanations based on the contraction of the rays.

- (1) Contraction of permanent rays (Heidenhain, '95, Kostanecki, '96).
- (2) Contraction of temporary rays (Boveri, Rhumbler *et al.*).

(*b*) Explanations based on the expansion of rays: Meves "Expansion theory."

(*c*) Explanations based on changes of surface tension (those which are based on the change of surface tension alone without discussing its cause are not explanations but mere restatements of fact):

- (1) Change due to the rays, which represent flows in the cytoplasm (Bütschli, '76, p. 414, Giardina, '02, pp. 500, 576).
- (2) Change due to the general currents, the rays being not important for cytodieresis (Platner, '86, Loeb, '95 a, b, Conklin, '99, '02, Bütschli, '00).

(*d*) Explanations based on the contraction of the peripheral plasmlayer (rays are of little use) (Flemming, '77, Ziegler, '98, pp. 43, 47, '01, p. 126, '04, pp. 552, 553).

(*f*) Explanations based on the general contraction of cytoplasm around the centres (rays are of little use) (Ziegler, '95, p. 70, Morgan '99, Gallardo, '02, p. 74, Teichmann, 03, Gurwitsch, '04, Bonnevie, '06).

VI. Formation of Polocytes.

In the living egg of *Cerebratulus lacteus* one observes at the animal pole a depression as soon as the first maturation figure reaches there (Kostanecki, '02, p. 272, cf. Rhumbler's polar depression, '96, p. 607). In case the egg is fertilized before the dissolution of the germinal vesicle the polocyte formation follows soon after the maturation figure comes to the animal pole. But otherwise the depression remains until the egg is fertilized. Immediately after fertilization a clear drop containing refringent chromosomes and destitute of yolk drops, flows out as though a portion of peripheral hyaloplasm gushed out through a hole. The spinning activity of the polocyte is beautiful as described by Andrews ('98) and C. B. Wilson ('00).

In sections of the normal egg accumulation of hyaloplasm is seen over the animal pole. As the polocyte bulges out, the chromosomes flow in, they being crowded at first in the narrow stalk of the polocyte and finally passing into it. No alveoles can be seen in the polocyte. The second one is formed in the same way. A mid-body is formed only between the second polocyte and the egg.[30]

As to the mechanism of the polocyte formation little has been discussed. The processes seem to be of entirely different nature from ordinary cell division. There are two types in the polocyte formation, namely, in one class the polocyte is formed before and in the other, after fertilization. In the latter, the first maturation mitosis stops at a certain stage and only after the entrance of the sperm is the polocyte formed. It is hardly necessary to enumerate the cases, in which the spermatozoon plays an important *rôle* in the reorganization and stimulation of cytoplasmic activity accompanying with it, *e. g.,* contraction, amœboid movements. An interesting experiment performed by Hertwig may, however, be worth mentioning here. He pricked the eggs of *Rana temporaria* with a glass needle, but nothing happened until they were fertilized. The extraovates are formed from the wounded eggs only upon fertilization

[30] The nucleus of the first polocyte is a compact chromatin mass, while that of the second is a vesicular one, the chromatic band with jagged surface coiling just beneath the nuclear membrane. Division of the first polocyte sometimes takes place.

('93, pp. 14, 15). This seems to show that the sperm causes the contraction of the egg.[31]

The action of the centriole upon the polocyte formation, on the other hand, should not be overlooked. The center seems to cause a sudden decrease of surface tension at the spot where the polocyte is to be formed, provided the cytoplasm be in the right state.

In the non-fertilized egg of *Cerebratulus lacteus* treated with a solution of magnesium chlorid a pointed protuberance resembling somewhat the entrance cone of the sea-urchin egg is sometimes formed at the animal pole. This unsuccessful attempt of polocyte formation may be due to untimely sinking of the maturation figure toward the center of the egg.[32]

The number and size of the polocytes may vary according to the physiological state of the egg. In some of the unfertilized eggs treated with a mixture of potassium chlorid and calcium chlorid (Professor Wilson's material) the polocytes have been produced.[33] The number and size of the polocytes thus produced showed great variation. Fig. 70 (Pl. IV) is an egg with a polocyte about five hundred times as large as the normal one. Fig. 71 and 72 (Pl. IV) are those with four and five polocytes respectively (Kostanecki describes the third polocyte, '02, p. 284).

VII. Summary.

A. Observations on the normal egg of *Cerebratulus lacteus*:

(1) Before the dissolution of the germinal vesicle the yolk granules are arranged radially.

[31]Hertwig adds to this experiment another possibility which is worth considering, *i. e.*, this result may be due to the gradual change in the nature of the oöplasm, since there is a considerable interval between the time of injury and fertilization.

[32]The solution used is 20/8 M $MgCl_2$ and sea water in equal parts; this is twice as strong as that used effectively for causing artificial parthenogenesis in the sea-urchin egg. The latter solution has no noticeable effect on the egg of *Cerebratulus*.

[33]The egg was put in a solution of 20/8 M $CaCl_2$ (210 cc.) + 20/8 M KCl (100 cc.) + Aq m (800 cc.) and gradually transferred into sea water. They were fixed after three hours.

(1) There are a few plasmosomes in the germinal vesicle. Each of them is usually found associated with a chromatin granule.

(3) The reduced number of chromosomes is 18 or 19; the somatic number, 36 or 38 (in *C. marginatus* the reduced number is 16 according to Coe and Kostanecki).

(4) Chromatin diminution is accomplished by the fading away of some of the chromatin granules at the prophase of the first maturation mitosis.

(5) The spermatozoon contains a centriole in the middle piece.

(6) The middle piece swells into a vesicle and the centriole escapes from it, giving rise to the centre of the sperm aster.

(7) The centrosome does not divide. The centriole divides in the centrosome. Each daughter centriole acquires its own centrosome, and the mother centrosome fades away.

(8) The centrosome of the fourth generation becomes that of the second cleavage.

(9) Crossing of rays takes place between the degenerating rays of egg-nucleus and sperm-rays.

(10) An antispindle figure, such as occurs in *C. marginatus* at the anaphase of the first cleavage, takes place very seldom. Such a figure may be an artifact.

(11) Sheath rays are formed surrounding the spindle at the anaphase of the cleavage mitoses.

(12) The first maturation figure remains unchanged for four or five hours if the egg is not fertilized.

(13) The sperm aster may pass unchanged from the alveolar into homogeneous plasm.

(14) A spindle may be formed between the maturation and sperm aster.

(15) The cytoplasm along the future cleavage plane is rendered less dense (partly by the nuclear fluid).

(16) Sheaf rays with the mid-body granules are formed at the telophase of the cleavage mitoses.

B. Observations on the abnormal eggs of *Cerebratulus lacteus*:

(17) In $CaCl_2$ eggs the chromosomes arise in the germinal vesicle in the form of thin threads.

(18) In unfertilized $MgCl_2$ eggs a protuberance is sometimes formed at the animal pole, as the maturation figure retreats toward the centre of the egg.

(19) In unfertilized KCl + $CaCl^2$ eggs the polocytes may be formed; the number and size of them vary a great deal.

C. Observations of other forms:

(20) In the blastomeres of the white fish (*Coregonus albus*) rays are found around the karyomeres.

(21) In the egg of *Pedicellina americana* the spindle of the first cleavage mitosis is bent at the telophase toward the animal pole (Dr. Dublin's observation).

D. General conclusions:

(1) The nuclear fluid is similar to hyaloplasm.

(2) The nuclear fluid is usually neither alveolar nor reticular but homogeneous.

(3) Diminution of chromatin (basichromatin) does not take place at the dissolution of the germinal vesicle.

(4) The centrosome is not a permanent organ, but is a temporary accumulation of centroplasm around the centriole.

(5) The centriole is a centre for the formation of rays.

(6) The size of the centrosome is proportional to that of the cell.

(7) The middle piece of the spermatozoon contains a centriole. The spermatozoon, therefore, carries a centriole into the egg at fertilization. The sperm centriole is not that of the cytasters produced by the egg.

(8) The cleavage centrioles are not new formations but those of sperm aster.

(9) The position of the division centrioles is determined by the egg-organization.

(10) Rays may be formed in homogeneous as well as in alveolar plasm.

(11) In fixed material fibrous and non-fibrous rays can be distinguished.

NOTE.—The writing of the present paper was finished on June 3, 1907, at the Zoölogical Laboratory of Columbia University. Very few alterations have been made since. Literature which has come to the author's notice since the above date is not referred to in this paper.

Zoological Institute Tokyo Imperial University, Japan, May 2, 1909.

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EXPLANATION OF THE PLATES.

Plate I.

1. An egg before the fading of the germinal vesicle. There are one large and nine small plasmosomes, each associated with a chromatin mass. Total preparation. × 333.

2. Nucleus of a blastomere at the two-cell stage. × 866.

3. The same at a little later stage; the nuclear membrane has been dissolved at the poles. Change in the chemical nature of the nuclear fluid. × 866.

4. Early prophase of the first cleavage mitosis, showing the darker portion of the rays in the nuclear region. × 866.

5. One large and two small plasmosomes with two chromatin masses. Auerbach's stain. × 2000.

6. Large plasmosome almost dissolved; disc-shaped precipitation granules are seen on the left hand side. × 866.

7. Basket-like residual mass of a larger plasmosome. × 866.

8. Four large and one small plasmosome. Near one of the large plasmosomes is a thread-like coagulum. × 866.

9. Small plasmosome with a chromatin mass. Reticular coagulation of the nuclear fluid. × 2000.

10. Small plasmosome with a chromatin mass; the former has noticeably decreased in size. × 866.

11. Small plasmosome consisting of two portions like the large one. × 866.

12. Larger plasmosome which has become hollow. One of the chromatin masses is embedded in it. × 2000.

13. A portion of the germinal vesicle of a $CaCl_2$-egg containing thread-like chromatin masses. × 866.

14. Prophase of the first maturation mitosis, showing four (or three) extra chromatin masses. Two long rays are reaching a chromatin mass. Combination of eight sections. × 866.

15. Equatorial plate of the first maturation mitosis seen *en face* consisting of 18 bivalent chromosomes. × 866.

16. The same consisting of 19 bivalent chromosomes. × 866.

NAOHIDE YATSU.

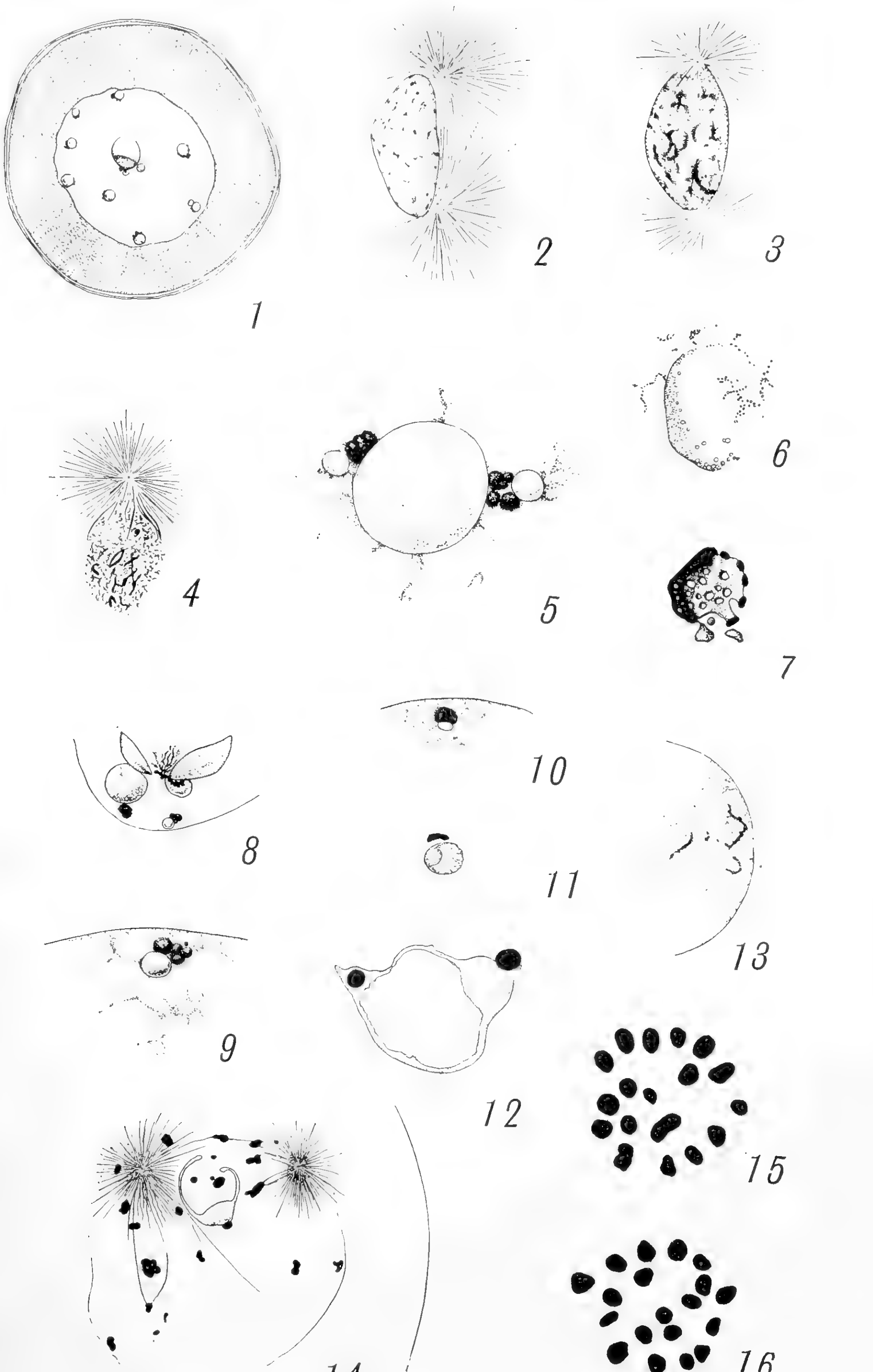

Plate II.

In Figs. 17, 18 and 21 only a portion of the tail is represented.

17. Young spermatozoon. Smear preparation. × 2000.

18. Young spermatozoon in which the middle piece has been formed. Axis through the middle piece. Smear preparation. × 2000.

19. Young spermatozoon with a long anterior process. The centriole can distinctly be seen in the middle piece. Smear preparation (well extracted). × 2000.

20. Full grown spermatozoon. Smear preparation. × 866.

21. Full grown spermatozoon more highly enlarged. Smear preparation (well extracted). × 2000.

22. Spermatozoon in the egg, already rotated. Thickening of the tail. × 2000.

23. Spermatozoon with rays appearing precociously around the middle piece. The rays are centering to the centriole. × 2000.

24. Spermatozoon with rays precociously developed. In this case the centriole has abnormally escaped from the middle piece. × 2000.

25. Spermatozoon in which an indentation has formed to mark the boundary between the head proper and the anterior process. × 2000.

26. Spermatozoon with a rounded head proper and a long anterior process. × 2000.

27. Spermatozoon with a rhomboidal middle piece. × 2000.

28. The same preparation further extracted. The middle piece has lost its outline, and the centriole has been brought out distinctly. Non-fibrous rays around the centriole should be noticed. × 2000.

29. Spermatozoon, in which the middle piece has become vesicular. × 2000.

30. Spermatozoon with middle piece a little more swollen. × 2000.

31. Spermatozoon with the first indication of rays. Notice a knob at the end of the tail. × 2000.

32. Middle piece with a pentagonal outline. × 2000.

33. The same preparation further extracted. The middle piece has lost its outline, the centriole alone remaining as a distinct body. Elongation of the tail. × 2000.

34. Middle piece surrounded by long rays. × 2000.

35. Centriole with ray-system escaped from the middle piece vesicle. × 2000.

36. Centriole just after its division. The middle piece vesicle is about to disappear. × 2000.

37. Sperm asters with new ray systems. No centrosome is present. × 2000.

38. Sperm asters with a synthetic spindle between them. × 2000.

39. Sperm aster with much centroplasm. × 2000.

40. Sperm aster more advanced. Two centrioles in a degenerating centrosome. The rays are about to disintegrate (only a portion of the rays are represented). × 2000.

NAOHIDE YATSU.

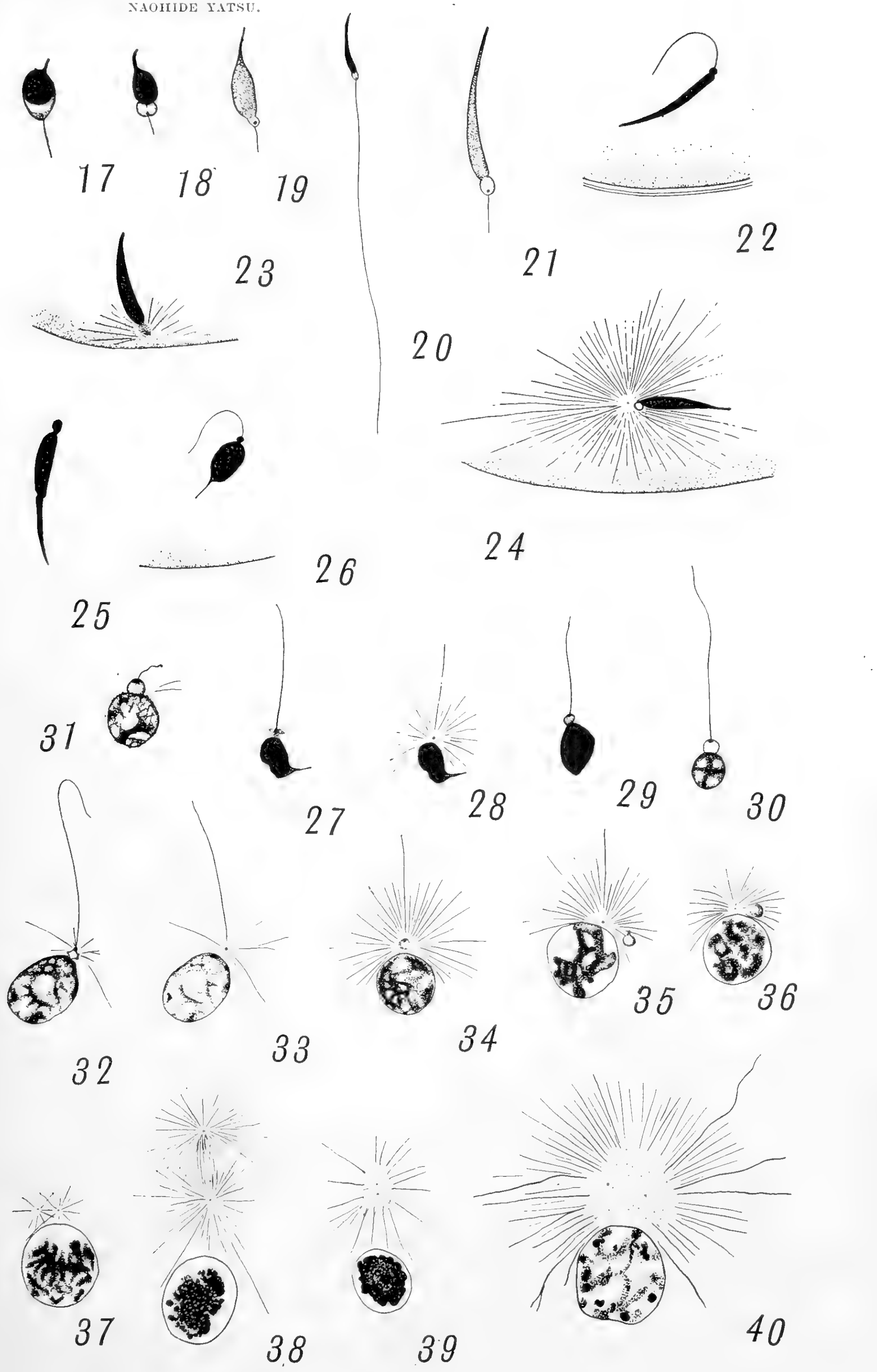

Plate III.

41. Two germ-nuclei immediately prior to their conjugation. The sperm-spindle lies between the nuclei. × 866.

42. Two germ-nuclei opposing each other. The sperm-spindle is behind the nucleus. × 866.

43-44. Two sections (not consecutive) of germ-nuclei fused. Two centrioles are present. Position of the centrioles in the degenerating asters. Small ray systems have appeared around the centrioles. × 866.

45. Two germ-nuclei opposing but not yet fused. A centriole (sketched in from the next section) has already acquired a new ray system. × 866.

46. Segmentation nucleus with the sperm-spindle on one side. × 866.

47. Centrosome containing two centrioles at a pole of the first cleavage figure. × 2000.

48. Centrioles separated farther from each other with two connecting fibres between them. × 2000.

49. Centrosome enlarging. × 2000.

50. Centrosome still enlarging, its alveolar nature becoming apparent. × 2000.

51. Centrosome with the centrioles connected with a single fibre. × 2000.

52. Centrosome, in which the centrioles have taken their definite position. × 2000.

53. Centrosome containing centrioles with new rays. × 2000.

54. Aster in a blastomere of a parthenogenetic egg of *Asterias*, showing the relation between fibrous and non-fibrous rays. × 1566.

55. Parasitic asters on a ray of the central aster of the first maturation figure. × 866.

56. Karyomere rays in a blastomere of *Coregonus*. Diastem along the equatorial plane. × 693.

57. Aster near the egg-nucleus in a parthenogenetic egg of *Asterias*. × 1566.

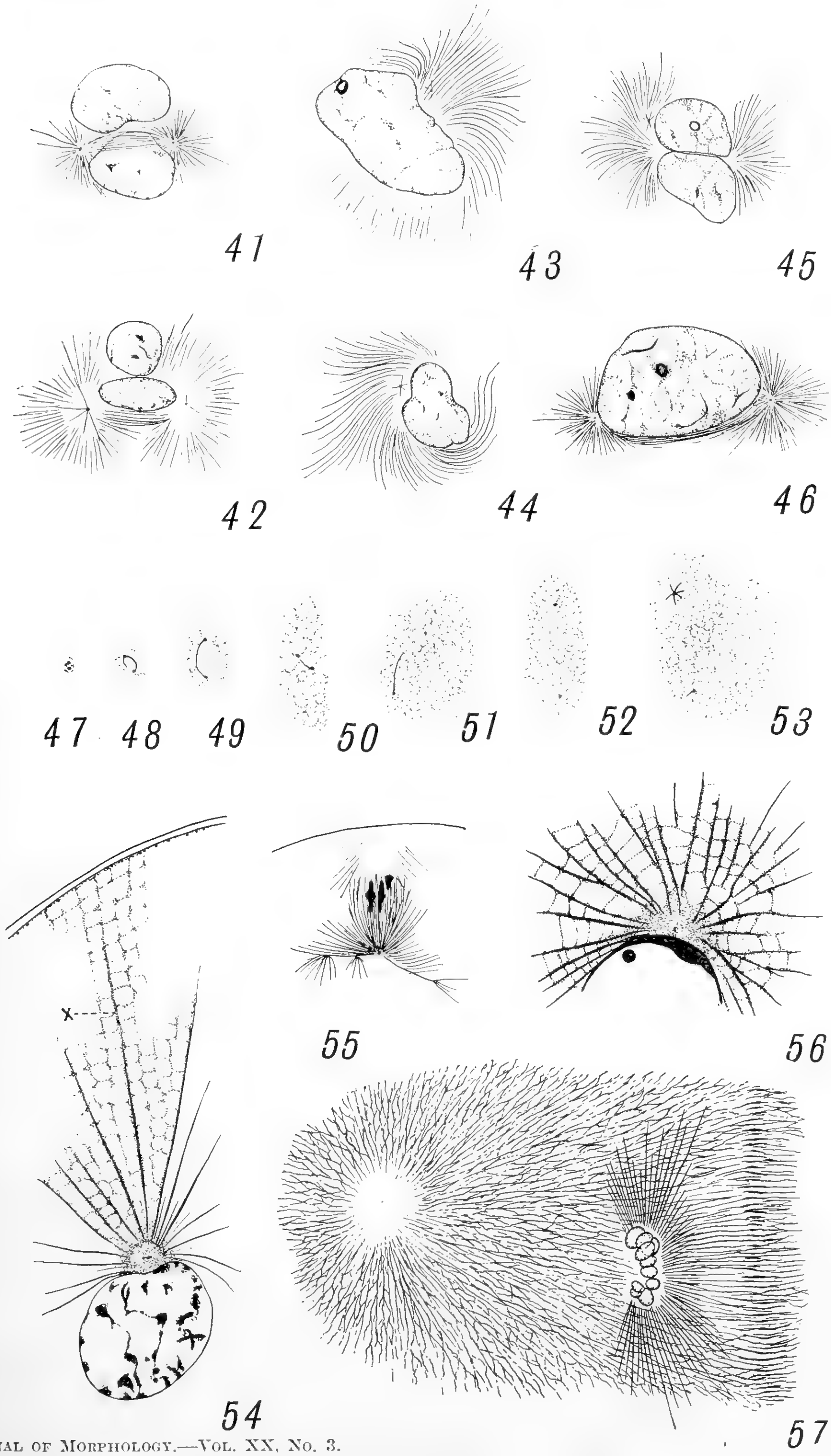
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Plate IV.

58. Central aster of the first maturation figure, showing archiplasmic differentiation of the rays. × 2000.

59. Sperm-aster in the nuclear area. In the next section is found the first maturation figure. × 866.

60. Rays of the maturation aster reaching chromatin masses. × 866.

61. Mitotic figure in an ether egg of *Asterias*, in which one of the asters has divided into two. × 526.

62. Sperm-asters approaching the first maturation figure. Some of the chromosomes are attracted towards the sperm asters. × 866.

63. Two cytasters in an ether egg of *Asterias*. × 1566.

64. Two asters in an enucleated blastomere of an ether egg of *Asterias*. × 1566.

65. Two asters connected by a spindle in an enucleated blastomere of an ether egg of *Asterias*. × 866.

66. Spindle formed between the central aster of the first maturation figure and the sperm-aster. × 866.

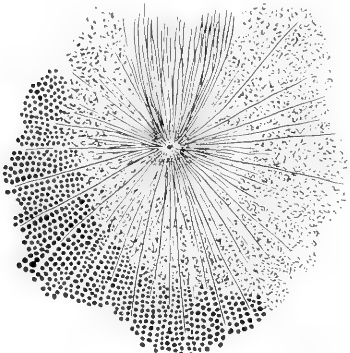
67. Spindle formed between two sperm-asters in a dispermic egg. × 866.

68. Sperm-nucleus approaching the egg-nucleus. The sperm-asters are behind the sperm-nucleus. One of the centrioles is under the nucleus. Fountain figure in the sperm-rays. Combination of four sections. × 866.

69. Crossing of rays between the sperm and egg-asters. Accumulation of the nuclear fluid between the asters. × 866.

70. First polocyte produced by $KCl + CaCl_2$ (unfertilized egg). × 866.

72. Five polocytes produced by $KCl + CaCl_2$ (unfertilized egg). × 866.



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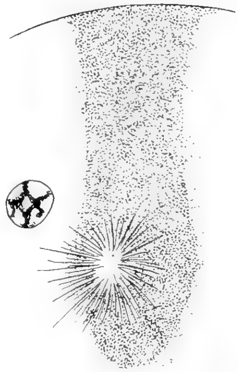

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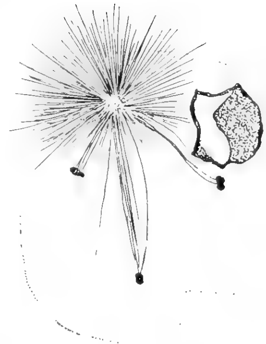

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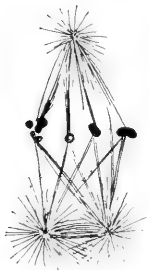

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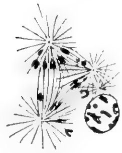

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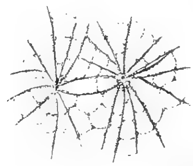

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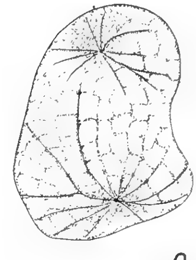

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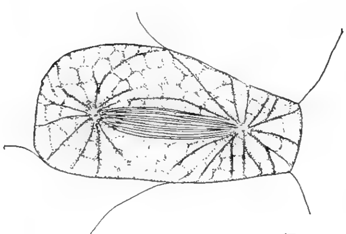

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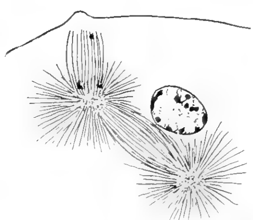

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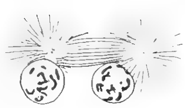

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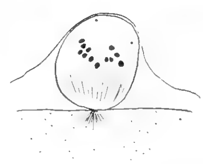

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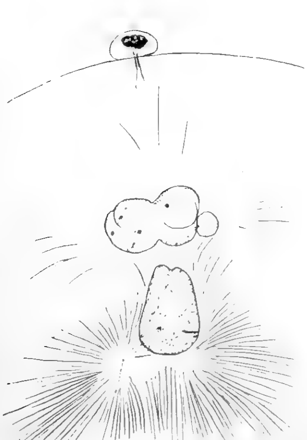

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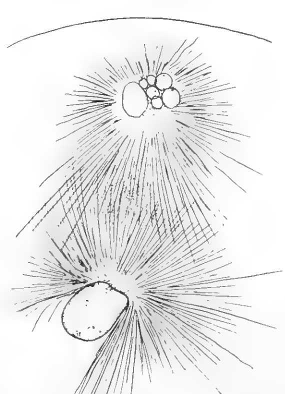

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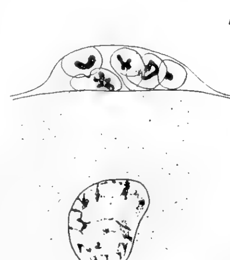

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STRUCTURE OF LIMULUS HEART MUSCLE.

WALTER J. MEEK.[1]

Since the physiology of the Limulus heart has been so carefully investigated and that organ has been used so successfully in attacking many physiological problems, it has seemed desirable that something should be known concerning its histology. The chief objection to applying any of the results obtained on this form to the vertebrate heart has been that Limulus is a representative of a group unique in the animal kingdom and well removed even from its nearest relatives. This fact makes it all the more desirable to investigate the heart musculature in this form and to find what similarity, if any, it bears to the vertebrate type.

Material and Technique.

Heart tissue from adult Limuli was used. The most of the material was prepared at Wood's Hole where fresh tissue was also examined. The hearts from some animals that had been shipped to Chicago were also used. These animals were in good condition and nothing atypical was found in the tissues.

A number of fixatives were used, including Carnoy's solution, Perenyi's fluid, acetic-sublimate, and Zenker's solution. Of these Zenker's proved by far the most serviceable and satisfactory. The hearts were split open, washed in sea water to free them from blood

[1]I take pleasure in thanking the Marine Biological Laboratory for material and also Dr. Bardeen and Dr. Erlanger, of the University of Wisconsin, for technical assistance. My thanks are also due Dr. Carlson, of the University of Chicago, for advice concerning the work.

and leucocytes, and then small pieces immersed in the fixative. The usual methods of embedding in paraffin and sectioning were employed. Sections were made from 3 to 6 micra in thickness.

Several different stains were employed, but iron hæmatoxylin followed by eosin was found to be the best for general study. The combination has been used largely by investigators and a comparison with their work therefore becomes easy. Orange G and fuchsin were frequently used as counter stains. In studying connective tissue distribution iron hæmatoxylin followed by Van Gieson's picro-fuchsin gave good results. A stain equally good and used with success was Mallory's connective tissue stain. Vanadium hæmatoxylin was also used in an attempt to demonstrate a sarcolemma. Fresh material was also treated with silver nitrate.

Fresh material was teased and studied in sea water or salt solution. Fresh tissue was also macerated in 20 per cent nitric acid, 40 per cent potassium hydroxide, 30 per cent alcohol, and saturated ammonium carbonate. Nitric acid is especially good as a macerating agent, since the preparation may be washed, passed into glycerine, and preserved. Material fixed in Zenker's solution and preserved in 70 per cent alcohol was also found to tease easily. These preparations could be stained with hæmatoxylin and eosin and then showed nearly all the details clearly.

General Morphology.

The heart of Limulus is a hollow sack 15-20 centimeters long and 1.5-3 centimeters wide. It is swung in the pericardial sinus by eight pairs of connective tissue ligaments, the alary muscles, which form a lateral support for the heart and extend to the pericardium with which they fuse. On its dorsal surface the heart has eight pairs of ostia. The ostia are narrow slits connecting the pericardial cavity with the lumen of the heart, and they are supplied with inward projecting connective tissue lips which make efficient valves. Leaving the organ are eleven arteries; four pairs of lateral arteries from the four anterior segments, two aortic arches, and one median aorta. The aortic end of the heart is provided with a strong connective tissue valve around which the heart musculature curves concavely

on the dorsal side. The posterior end is attached to the carapace by a broad sheet of connective tissue. The outer surface of the heart looks as if it were longitudinally striated, an appearance which is due to a layer of elastic tissue strands.

Microscopic Histology.

In cross section the heart is somewhat triangular in outline and may be seen to consist of three layers. (See Fig. 1.) The median layer (Fig. 1, b) is a fine, dense, connective tissue support, termed the basement membrane (Patten and Redebaugh, 1899). Outside of this is the longitudinal layer of elastic tissue fibers mentioned above. Inside the basement membrane is the muscular layer which consists of branching, anastomosing strands, arising from the basement membrane and running mostly in a circular direction. The muscular layer is thickest at the angles of the heart and thins out over the aortic valve. It is with this layer that we are most concerned.

By reference to Fig. 1 the general characteristics of the muscular heart wall can be understood. Trabeculæ of striated muscular tissue arise from the basement membrane, branching and anastomosing until a spongy heart wall is formed. No membrane limiting the lumen corresponding to the endocardium of the mammalian heart is present. In only one region is the lumen lined and that is at the aortic valve where for a short space the muscle layer lies between two connective tissue layers, the basement membrane above and the tissue of the valve beneath.

The blood circulates freely around the strands of the heart, passing into all the interstices and crevices, thus bathing the entire musculature. This arrangement for securing nutriment seems to be quite sufficient. A small amount of connective tissue appears between the trabeculæ, especially in the thicker parts of the heart wall. This can easily be traced back in origin to the basement membrane. It would seem that capillaries might enter along with this tissue, but as far as can be determined they do not do so, unless it be in the deeper parts immediately adjacent to the basement membrane.

A small fragment of the heart wall macerated in 20 per cent nitric

acid and teased with needles gives such pictures as represented by Fig. 2. The trabeculæ are at once apparent. They are of all sizes and run for the most part circularly around the lumen of the heart. These strands branch freely in all directions and join neighboring strands. Preparations teased in this way show even more plainly than the cross section described above, that the heart is a great network of muscle, whose strands form a spongy wall or a thick solid one according as they are mingled together. In the heavier heart walls the strands branch with rather acute angles, and in life the meshes between are no doubt mostly potential spaces. In the parts near the lumen the meshes are large. Often many trabeculæ fuse and form large sheets of muscle, which are really only large trabeculæ, since they again divide and the parts reunite with neighboring strands. (See Fig. 3.)

On teasing these trabeculæ with fine needles it is seen that they tend to split into smaller strands along lines fairly well determined at least for short distances. These smaller strands which may be regarded as the real heart fibers branch and anastomose with each other within the primary trabeculæ. This arrangement is particularly visible in tissue macerated in Zenker's solution, stained, and examined in glycerine under high power. Fig. 4 shows this arrangement semi-diagrammatically.

So far as can be determined neither the trabeculæ nor the secondary anastomosing strands composing the larger trabeculæ, end freely. Marceau (1904) has described such a condition in the lower vertebrates. He finds a large number of heart fibers ending freely among the trabeculæ in the Fishes, Batrachians, Saurians, and Ophidians. In the Chelonians and Crocodilians the free endings are quite rare, and in the higher vertebrates they do not occur at all. In teased specimens of Limulus heart muscle the endings found are always such as those shown in Fig. 2. The smaller branches here represent the heart fibers and it is very apparent that the blunt ends are due to transverse rupture by the needles. Conical or filiform terminations, which would be the characteristic shape or natural endings, have not been found. If they do occur they must be somewhat rare. In this particular the heart of Limulus is like the higher vertebrates.

The structure thus far described has been made out by teasing in macerating fluids. For more definite work stained preparations are necessary. The chief interest centers in cross and longi sections of the trabeculæ. Such sections determine the accuracy of the ideas already obtained and also give the structure of the fibers making up the trabeculæ.

When a specimen is sectioned parallel with the long axis of the heart most of the trabeculæ are seen in cross section. The field is filled with oval, circular, triangular, or short ribbon-like areas. These are the trabeculæ, and it is apparent that they vary greatly in size and shape. The smallest are about 12 micra, and the largest observed were about 90 micra in diameter. Near the basement membrane the areas are closely appressed, while toward the lumen there is considerable space between. The arrangement is irregular and the whole appearance is what one might expect from cutting across strands that form a spongy heart wall.

Figs. 5, 6, and 7 show four of these trabeculæ in cross section. The greater portion of each area is mostly filled with the cross sections of the contractile fibrils which appear as black dots. These have no very definite arrangement. In some cases they seem to have a reticulated appearance, as in Fig. 7. In others they are in more solid masses, as in Figs. 5 and 6. Only in the smallest is the strand ever completely filled with fibrils. Between the fibrils sarcoplasm is of course present. Clear areas are nearly always present in the cross sections. Some of these may be due to vacuolization produced by the fixative, but a certain number probably represent the poorly staining protoplasmic columns of the muscle fibers. In these columns are found the nuclei of the muscle fibers. Surrounding the fibrillar area is a thin cylinder of protoplasm which is bounded externally by a fine but perfectly definite line. Within this boundary and lying in the protoplasmic cylinder are found nuclei which in cross section resemble those found in the protoplasmic cylinder. (See Fig. 7 for illustration of these details.)

The number of nuclei in the clear areas of the fibrillar portions is variable; there may be of course none, and there may be as many as five or six in the larger trabeculæ. When more than one nucleus

appears at a given level each one probably belongs to a separate so-called fiber. The territory of each fiber is, however, impossible to determine, a fact that is due to the irregular arrangement of the fibrils. Fig. 6 illustrates this point. Here two nuclei appear and one would judge that at least two fibers composed the trabecula, but it would be impossible to divide the fibrillar area into two very definite regions. This fact is due to the free branching and anastomosing of the fibers within the primary trabeculæ. Since these secondary strands have no well defined boundaries, yet branch and fuse with each other, the heart muscle must be considered a syncytium. In a syncytic structure one can scarcely speak of definite cells or fibers and this is plainly borne out by the confused fibrillar areas in the cross sections of the trabeculæ. The contractile elements form strands which branch and fuse. These strands may be called fibers or cells for convenience, but they must not be confused in structure with such fibers as we have in skeletal muscle.

In longi sections, cross and longi striation is well brought out by the iron hæmatoxylin method. As in the heart muscle of all other forms it is evident that each contractile fibril is continuous throughout the entire musculature, ending only where the muscle fiber itself takes its origin. The clear areas of the cross sections now appear as longitudinal clefts mostly filled with protoplasm. In these lie the muscle nuclei. Fig. 8 shows a trabecula illustrating these points. These muscle nuclei are long and narrow in diameter, with edges which curve out toward the membrane of Krause. There is a scant chromatin network and at least one nucleolus is usually visible. The fibrils are grouped into strands, which illustrates the inner syncytic structure already discussed. Outside of these strands is again seen the cylinder of protoplasm and outside of this the fine linear boundary (c and f in Fig. 8). The peripheral nuclei are now seen to be oval in shape. They equal the muscle nuclei in diameter, but otherwise do not resemble them closely. The boundary membrane appearing as a fine line (*f* in Fig. 8) is attached to the Z line of the muscle fiber, the membrane of Krause, and for this reason it appears in a series of festoons rather than a straight line. It has proved quite a task to decide what relation this outer protoplasmic

cylinder with its limiting membrane and peripheral nuclei bears to the contractile portions within. The boundary line at first sight is strikingly similar to the membrane Heidenhain figures as a sarcolemma (1901) in human heart muscle. There is the same protoplasmic area just outside the outermost fibril and the membrane arches in festoons to meet the Z line. This gives the same effect that McCallum describes in mammals (1897), where he says the rounded edge of the sarcoplasmic discs makes up the edge of the fiber. The presence of nuclei just beneath this membrane seems opposed to the view that a sarcolemma is present unless it is assumed that there are two kinds of nuclei present in the fiber, one being at the periphery to attend to the growth of the cell. The facts are without question, it being merely a matter of interpretation. Of course if the trabeculæ were considered as having a sarcolemma the strands within could no longer be considered as the real heart fibers. The structure would still be a syncytium, but the significance of the secondary syncytium within the trabeculæ would be changed.

Most observers, however, agree that the lower forms do not have a sarcolemma (Renaut et Mollard, 1904), the heart fibers being bare. The simpler explanation would be that the trabeculæ are provided with a connective tissue sheath. This was tested by using standard connective tissue stains, and it was found that the limiting membrane stained blue with Mallory's and pink with Van Gieson's. Van Gieson's was not entirely decisive, however, since the Z line also took the same color. By keeping sections twenty minutes in the acid fuschin solution of Mallory's stain and then reducing the time in the aniline blue solution to about three minutes, it was found that the limiting membrane always stained blue, while Krause's membrane often took a bright purple color. This differentiation in color would indicate that the two structures were different. Heidenhain (1901) found that vanadium hæmatoxylin stained the sarcolemma and the Z line both blue, connective tissue taking a much lighter bluish color. In Limulus sections vanadium hæmatoxylin stained the basement membrane a faint blue and the muscle substance an orange, which seemed to indicate that the stain was ripe and working properly. The membrane in question, however, seemed little if at all

affected by the stain, and this is what one would expect if the structure were connective tissue. Being thin it would stain so faintly that it would make but little impression.

These results enable us to formulate our conception of the heart musculature. The heart wall is made up of branching, anastomosing trabeculæ which are individualized by connective tissue sheaths. Within these trabeculæ are the naked strands of muscle which also branch and fuse and thus form a true heart syncytium. Possibly the connective tissue sheath takes the place of a sarcolemma by functioning as a dialyzing membrane for the exchange of the nutriment. If it is related in any way to the development of the sarcolemma in higher forms it must be noted that the relation here is to the trabeculæ and not directly to the fibers within.

In Limulus then we have the same general conditions that are found in the lower vertebrate heart. There are no free endings of muscle fibers, but these even disappear with the higher reptiles. The connective tissue sheath seems more closely appressed than in the case of most forms figured by Marceau. With these two differences, neither of which can be fundamental, the heart muscle can scarcely be told from that of the lower vertebrates. To convince oneself of this it is only necessary to compare Marceau's figures of the lower vertebrates, particularly the fishes, frog, and turtle, with those figured for Limulus.

Physiologically the important fact brought out by a study of the Limulus heart musculature is that it is a syncytium practically indistinguishable from that of the vertebrate heart. Carlson (1904) has shown conclusively that the normal myocardium of Limulus is incapable of physiological conduction. When the nerve ganglion is removed the muscle responds to a local stimulus by a local contraction and there is no conduction whatever of the contraction to a more distant part of the organ. We have here then a heart, syncytic in structure, with a protoplasmic continuity throughout, which nevertheless does not conduct under normal conditions. This fact, that muscular continuity may be associated with the absence of muscular conduction, renders invalid the argument drawn from the syncytic structure in favor of the myogenic theory of conduction in the vertebrate heart.

A few more points may be of interest. The trabeculæ take their origin from the basement membrane. Here and in the connective tissue guarding the ostia are the only places where fibers have been found to end. The ending is not often conical, as noticed in skeletal muscle. The fibers rather fray out and the connective tissue inserts itself between the fibrils. The connection is thus one of fibrils to connective tissue rather than that of an entire strand.

The trabeculæ are often separated by narrow clefts into which the connective tissue sheath inserts itself, as may be seen in Fig. 9. This makes the trabeculæ on the average rather narrow in diameter. The function of the sheath in Fig. 9 may be a support, since the cross striation shows that the fibrils are not in synchrony.

Bands of Eberth are of course not found in the Limulus heart. These structures do not appear in man until after birth and are not found lower in the animal kingdom than in the birds. Their absence in Limulus is therefore of no significance.

Summary.

The heart musculature of Limulus is a double syncytium. It consists of branching, anastomosing trabeculæ individualized by connective tissue sheaths, within which heart fibers branch and anastomose, thus forming a continuous network of contractile tissue.

The heart tissue studied agrees with all heart tissue in these fundamental facts.

1. It has the regular cross striation.

2. Contractile fibrils are continuous throughout the musculature, ending only where the fibers take their origin.

3. The trabeculæ are provided with a peripheral covering which may serve as a dialyzing membrane.

4. The heart muscle is a syncytium.

The fact that the heart musculature is syncytic would show that a continuity of musculature is not decisive evidence for the myogenic theory of conduction.

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EXPLANATION OF PLATE I.

Fig. 1. Semi-diagrammatic portion of entire heart wall. a—branching muscular layer. b—Basement membrane. c—Elastic tissue.

Fig. 2. Trabeculæ teased in 20 per cent nitric acid. Drawn with camera lucida. Leitz Ob. 6, Oc. 4. a—Blunt end of small strand.

Fig. 3. Large flat trabecula. Teased from 20 per cent nitric acid and drawn under camera lucida as above.

Fig. 4. Semi-diagrammatic. Constructed from thick paraffin section. Anastomosing trabeculæ are shown with anastomosing strands of muscle within. a—Sheath around trabeculæ. B—Strand of contractile tissue. d—Nucleus of contractile strand. c—Nucleus of sheath.

WALTER J. MEEK.

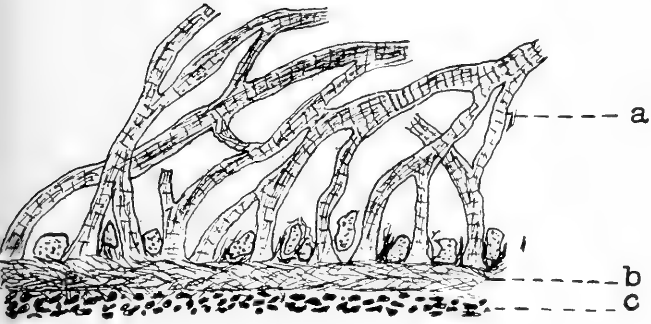


FIG. 1.

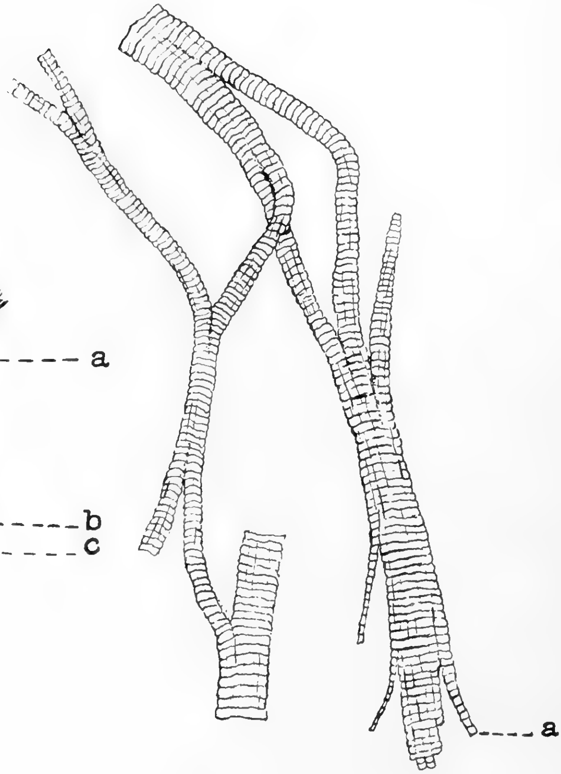


FIG. 2.

FIG. 3.

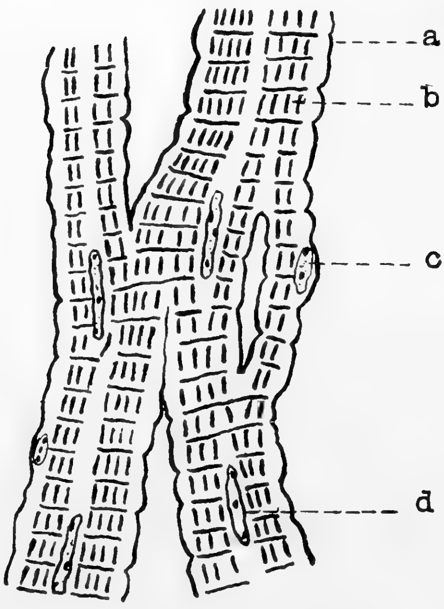


FIG. 4.

EXPLANATION OF PLATE II.

FIG. 5. Cross-section of two small trabeculæ. Stained in iron hæmatoxylin and followed by Van Gieson's. Leitz Ob. 12, Oc. 3. Camera lucida. $\times$ 1,000. Lettering the same as Fig. 7.

FIG. 6. Cross-section of a trabecula. Stained in iron hæmatoxylin and followed by Van Gieson's. Leitz Ob. 12, Oc. 3. Camera lucida. $\times$ 1,000. Lettering the same as Fig. 7. Shows two nuclei at same level, each in its axis of protoplasm.

FIG. 7. Cross-section of a large trabecula. Stained in iron hæmatoxylin followed by eosin. Leitz Ob. 12, Oc. 3. Camera lucida. $\times$ 1,100. a—Nuclei of connective-tissue sheath. b—Connective-tissue sheath around trabecula. c—Protoplasm within sheath. d—Nucleus of muscle fiber. e—Axial protoplasmic region in which nuclei are embedded.

FIG. 8. Longi section of a trabecula. Iron hæmatoxylin followed by Van Gieson's. Leitz Ob. 12, Oc. 3. Camera lucida. $\times$ 1,100. f—Connective-tissue membrane. g—Strand of contractile fibrils. Other letters the same as in Fig. 7.

FIG. 9. Longi section stained with Mallory's connective-tissue stain. Color shown as in preceding figures. Leitz Ob. 12, Oc. 3. $\times$ 1,100. a—Cleft dividing the trabecula into which the connective tissue sheath has entered. The appearance is similar to what Heidenhain figures as the "daughter sarcolemma."

FIG. 10. Longi section. Iron hæmatoxylin followed by eosin. $\times$ 1,100. a—connective-tissue sheath which persists between the fusing trabeculæ until the cross striation becomes synchronous.

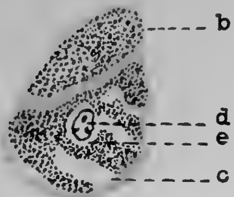


FIG. 5.

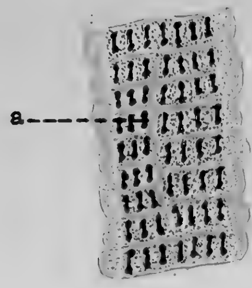


FIG. 9.

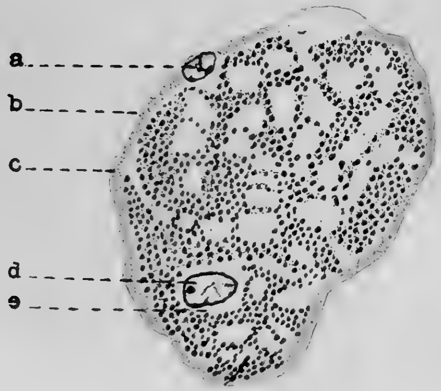


FIG. 7.

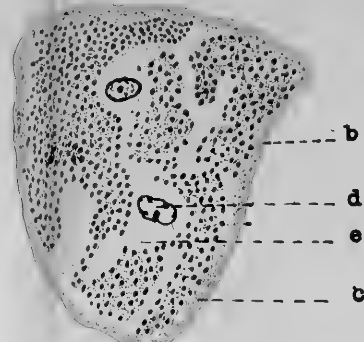


FIG. 6.

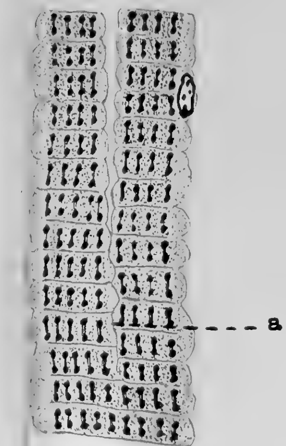


FIG. 10.

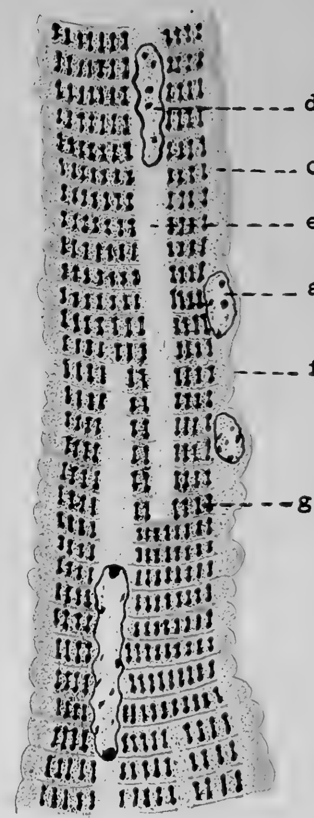


FIG. 8.

HISTORY OF THE PROCEPHALIC LOBES OF EPEIRA CINEREA.

A Study in Arachnid Embryology.

AVERY E. LAMBERT.

TABLE OF CONTENTS.

I. Historical Statement.

Among the objects to attract the attention of the earliest workers in the field of invertebrate embryology, the study of the Arachnids occupied an interesting and important place.

The results of work done on the Araneina, however, have been very general in their character; few investigators have undertaken special problems relating to the subject. Within a few years several excel-

lent papers have been published which show that the trend of observation, in recent years, has been in that direction.

The earliest studies on the embryology of the Araneina were undertaken before the technical methods, now employed in such investigations, were known. For this reason, the earliest papers on the subject have little value for us apart from their historical interest.

The earliest paper to deal distinctively with the development of the spider was published by Herold (15) in 1824. In 1862 Claparedé (8) published the results of his studies on the earlier stages in the development of the spider. His observations were made with surface preparations only, and consequently failed to throw light on the segmentation of the nucleus and the formation of the germ layers.

He succeeded, however, in carrying his studies of the development of the external form of the embryo up to the period of reversion. He observed and figured the caudal thickening, to which he gave the name "primitive cumulus." This term has not been consistently applied, by those who have subsequently treated the subject, to the accumulation of cells which form a slight elevation in the caudal region of the embryo; but has been more commonly used to designate the first accumulation of cells which occurs in the middle region of the blastodisc.

The division of the yolk of the spider's eggs into columns, and the subsequent arrangement of these columns in the form of "rosettes," was correctly reported by Ludwig (24) in his study of the formation of the blastoderm of spiders' eggs, published in 1878. He also noted the fact that the blastoderm arises at one end, instead of covering the entire egg.

In 1878, Barrios (3) published the results of his study of the development of the spider in which he called attention to what he termed a "limuloid" stage, referring to the form of the embryo previous to the period of reversion.

Up to this time investigators had to content themselves with the study of the external form of Araneid embryos. Balfour (1), who first applied the section method to the study of Arachnid embryology,

published a paper in 1880 which contained several important contributions to the knowledge of the subject.

Owing to his failure to obtain sections of the pre-blastodermic stages he was led to attribute the formation of the mesoderm, in part, to cells which, he believed, migrated into this layer from the yolk. He observed, however, and correctly reported, that a portion of the mesoderm is derived from the blastoderm.

Schimkewitsch (38), in a paper published in 1887, maintained that all of the cells derived from the division of the segmentation nucleus do not migrate to the surface of the egg, but some remain in the yolk and contribute to the formation of the endoderm; this conclusion later researches have failed to confirm. But this author was able to demonstrate that the rostrum, in spiders, arises as a pair of prominences on the anterior margin of the cephalic plate, these prominences uniting later to form the upper lip.

Morin (30) in a brief paper which appeared in 1886 followed the whole course of the development of the spider. In his study of the fate of the segmentation nucleus, he found that the nuclei, resulting from its division, rise to the surface of the egg, the greater number of them participating in the formation of the blastoderm. According to this author, the cells which appear in the yolk, after the formation of the first cellular layer, are not derived from cells that have been retained in the yolk, as had been stated by previous authors, but from the blastoderm itself.

The two most important contributions to the study of the embryology of the spider, and in many respects the most satisfactory, are those which were made by Locy (23) in 1886, and Kishinouye (19) in 1890. Locy made a complete study of the embryology of *Agalena naevia* by the section method. He established the fact that a depression exists in the middle region of the primitive cumulus, beneath which the multiplication of cells takes place very rapidly. Because of the relation which this depression bears to the mass of rapidly proliferating cells, he interpreted it as representing the blastopore.

The most important part of Locy's work is that which relates to the development of the anterior median eyes. He was able to show that these eyes arise by means of an infolding which causes an inver-

sion of the optic elements; the eyes becoming, by this means, a three-layered, instead of a two-layered, structure. This fact had been pointed out previously by Patten (32), in a more general paper on the eyes of Mollusks and Arthropods.

Kishinouye's paper is one of the most important among the later contributions to the subject. By his observation that the division of the segmentation nucleus is accompanied by a division of the yolk into parts corresponding to the number of the products of nuclear division, he has been able to throw important light on the segmentation of this class of eggs. He also found, with regard to the body cavity of the spider, that the original cœlom disappears except that portion which is enclosed in the heart, constituting the lumen of that organ, and in the stercoral pocket; the body-cavity of the adult spider being a secondary acquirement. The development of the respiratory apparatus he associates with the first two pairs of abdominal appendages; the remaining abdominal appendages being involved in the formation of the spinning mammillæ.

His description of the origin of the anterior—or as he calls them, the posterior—median eyes agrees with the observations of Patten and Locy. He describes the other pair of median eyes and the lateral eyes as originating in simple thickenings of the ectoderm in the optic area; a radical departure from the conditions of origin and development of the so-called accessory eyes as stated by Locy.

While St. Remy (39), in his excellent monograph on the nervous system of Arthropods, has given an accurate description of the conditions in the adult brain of the spider, either on account of the difficulties presented by the problem, or for want of interest in it, very little work has been accomplished, in relation to the special problems of development, on the Araneid brain. Neither Balfour, Locy, nor Kishinouye, have given satisfactory accounts of the origin and development of the cephalic lobes.

Balfour found that the brain originates as a thickening of the cephalic plate, and that it presents a segmented condition. But he failed to state whether he regarded the segmentation of the cephalic plate as having any special significance. He followed the development of the brain through to its separation from the ectoderm, and

found that, in its later condition, it consisted of a principal cerebral mass which is connected with the sub-œsophageal ganglia by a pair of commissures consisting of the two halves of the cheliceral segment; the sub-œsophageal mass consisting of the remaining thoracic ganglia.

Locy, in his paper on the development of *Agalena nævia,* failed to add much to the existing knowledge of the cerebral lobes; although he successfully followed the development of the anterior median eyes which, in their formation, are intimately associated with the development of the anterior lobes of the brain.

Kishinouye's account of the development of the brain of the spider, while in some respects more satisfactory than that given by his predecessors, still leaves much to be said. He states that the rudiment of the central nervous system is laid down very shortly after the formation of the germ band, and that the ectoderm of the cephalic plate is broken up into ridges by the formation of successive transverse thickenings which are continuous with similar thickenings that appear later on the inner margins of the lateral bands of the embryo. These thickenings, which appear in the ectoderm of the cephalic plate, indicate the beginning of the ganglia of the brain. Those which appear on the lateral halves of the ventral plate form the rudiment of the nerve cord.

Balfour, Locy and Kishinouye recognize the relations which the crescentic invaginations, which appear on the anterior margin of the cephalic plate, bear to the formation of the anterior vesicles of the brain. They are able to account for the formation of the lateral vesicles from similar, though smaller, invaginations which appear on the lateral margins of the plate. Kishinouye states that the anterior vesicles form the principal mass of the brain, but he failed to relate them, as Locy does, with the development of the anterior median eyes.

Patten (34, 35, and 36) called attention to the significance of the appearance of pre-oral segments in the cephalic plate of Arthropods, and pointed out that such segments were similar in character to those which were post-oral in position and bore appendages. He was able to show that the segments of the cephalic plate were divided by distinct thickenings of the ectoderm into cerebral and optic ganglia,

a condition which he first observed in *Acilius,* and found afterward in a more marked degree in scorpions and spiders. He was led to homologize the thickenings on the inner margins of the pre-oral segments, the cerebral ganglia, with the thickenings which occupy a similar position on the inner margins of the segments of the lateral bands, and which form the neuromeres which later develop into the nerve cord.

According to Patten, the vesicles formed by the anterior and lateral invaginations do not furnish the principal mass of the brain, but form important lobes instead which become associated with the optic tracts.

In his discussion of the development of the brain of scorpions, this author pointed out the fact that the entire area of the fore-brain is covered by a fold which progressively extends inward from the periphery of the lobes. This fold, he found, bears a most important relation to the formation of the median eyes, and also to what he calls the "vesicle" of the fore-brain.

The origin and development of the eyes have formed one of the most important subjects of investigation in Arachnid embryology, and furnish a problem which is closely associated with the study of the development of the brain.

The first contribution of importance to our knowledge of the subject was made by Grenacher, whose paper was published in 1879. Graber (13) immediately followed with an account of the morphology of the eyes of Arachnids, in which he failed to accept Grenacher's results.

Patten (32), in 1886, called attention to the fact that the median eyes of spiders consist of three layers, an outer, or corneal, a middle or retinal, and an inner or post-retinal, layer. He also pointed out that, in consequence of its manner of formation, the retinal layer became inverted so that its elements presented the same relation to the direction of the rays of light that we find in the vertebrate eye, a fact which was afterward established by Locy and Schimkewitsch, who made a careful study of their mode of development.

These observations were confirmed by Mark (25) in 1887, who discussed at length the morphological derivation and relations of the eyes of Arthropods.

Patten, in 1898, in his paper on color vision, has given a more detailed account of the remarkable structure of the retinal elements in the eyes of *Lycosa,* calling attention more especially to the relation the form of the retinal cells bear to their position in the head and to the direction of the rays of light falling upon them.

II. Materials and Methods.

In the following observations use was made of the eggs of *Epeira cinerea;* all of the materials being collected in, or about, Hanover, New Hampshire. This spider is one of the largest to be found in northern New England, which, together with its habit of infesting houses and barns where, as a general thing, its nests are made in easily accessible places, and the fact that the eggs are relatively large and are, therefore, easily handled, renders this material well adapted for embryological work.

The eggs are bound in a firm yellow mass in the cocoon, being held together by a kind of cementing substance which is probably secreted during their passage through the oviducts from the walls of the duct. They can be easily removed from the cocoon by cutting its walls away with scissors, and carefully manipulating the cut edges with forceps. Considerable care has to be exercised in isolating the eggs, as the membranes are easily ruptured. By cautiously forcing them out of the mass with needles, the majority of them may be removed without injury. This process is aided by their natural elasticity which is very great.

Three methods were employed in killing and fixing the eggs after they had been separated. The first was to plunge the eggs into water heated to 70°-80° C., and afterwards transferring them to 95 per cent alcohol in which they were hardened. Then they were placed in 70 per cent alcohol in which they were preserved. This is the method employed by Locy and Kishinouye. Unfortunately, however, it did not give good results except in the older stages; the yolk, in the earlier stages, showing a tendency to crumble and fall to pieces.

The second method was to kill the eggs in hot Perenyi's fluid, the eggs being left in the fluid from twenty to thirty minutes, or long

enough to ensure thorough penetration; after which they were passed through the different grades of alcohol to 95 per cent for hardening. The results obtained by this method were not entirely satisfactory, as eggs in both the younger and older stages showed a tendency to collapse. In those cases where collapse did not occur the preparations gave excellent results.

The third, and most successful method for all stages, was to plunge the eggs into picro-sulphuric acid (Kleinenberg's formula) heated to 70°-80° C., in which they were allowed to remain until the acid had cooled. They were then passed through the grades to the 95 per cent alcohol from which, after hardening, they were returned to the 70 per cent for preservation. By placing the specimen bottles on a water-bath at 50° C., the stain left the eggs more rapidly than at the ordinary temperature of the room, thus obviating one of the objections to the use of this reagent.

Fixing the eggs in hot fluids is of advantage in that it insures instant coagulation of the protoplasm, the rapid penetration of the reagent, and also helps to distend the closely-fitting egg-membranes. This makes the removal of the egg-membranes, which is necessary when the embryos are to be treated for surface preparations, a comparatively easy matter.

In preparing the embryos for study I have used a modification of Patten's method of staining and mounting. This method for obtaining permanent, detailed surface views of Arthropod embryos was first used in the preparation of the eggs of *Acilius, Blatta, Limulus* and *Buthus,* by Patten in 1888. It has been made use of since by other investigators with only minor modifications.

The method consists of the emersion of the naked egg, or embryo, in a strong stain for a few seconds if the staining of only the superficial layers is desired, or for a longer period in a more dilute stain if the penetration of the deeper cell layers is sought. By using strong, nuclear stains the yolk and cytoplasm of the cells absorbs but little of the coloring matter, and this may be readily removed by the use of acidulated alcohol, the result being that the surface contours of the embryo, and the distribution of the nuclei, are shown with the utmost clearness. The final mounting may be made in balsam or damar.

After the embryo has become invested with a chitinous cuticle, the process of staining is much slower; the cuticle being penetrated with difficulty. It is then necessary to keep the embryo in the stain several hours, or even for a day or two; this is followed by a slow decoloration with acid alcohol until all traces of the extra-nuclear stain have been removed.

For the preparation of the embryos the investing membranes were removed with needles, and the eggs allowed to remain in acid hæmalum (Mayer's formula) from thirty seconds to a minute, in which time only the nuclei of the outer layers were affected by the stain. For staining the deeper layers the hæmalum was diluted one-half, the eggs remaining in it from fifteen to twenty minutes. By means of this latter method the more deeply lying portions of the embryo can be distinguished, especially in those areas in which there is a rapid proliferation of cells.

Borax carmine was also used, giving very satisfactory results. In using the carmine some excess of stain is sure to appear both in the plasma and yolk. This has to be removed with acid. In the older stages the acid causes the yolk to swell, frequently splitting the embryo, and thus rendering the preparation useless.

Very early stages are difficult to handle on account of the closeness with which the membranes cling to the egg, making it almost impossible for them to be removed without injuring the underlying parts. For the examination of these stages resort was made to a method suggested by Prof. Patten, and which he had employed successfully in the study of the eggs of *Patella*. The living egg was placed on a glass slide in a few drops of glycerine, to which a drop or two of concentrated acetic acid had been added. After a few minutes the egg-membranes are penetrated by the acid and glycerine, and become sufficiently clear to enable one to distinguish the cells and their nuclei on the surface of the egg. This method is an excellent one for determining the stage in which the eggs are found before proceeding with the fixation.

III. Origin of the Germ Layers and Formation of the Cephalic Plate.

The eggs of *Epeira cinerea* are slightly elliptical and of a golden yellow color. They are a little less than a millimeter in diameter. The greater part of the egg consists of masses of yolk, separated from one another by thin sheets of protoplasm which radiate from the center of the egg. These protoplasmic sheets unite above the surface of the yolk where they form a thin layer called the periplasm.

The entire egg is surrounded by two membranes, an inner, vitelline membrane, which lies close to the periplasm, and an outer membrane, the chorion. The vitelline membrane is thin, delicate, and transparent. The chorion has a tougher texture, the egg being seen through it with difficulty. Both membranes are supposed to be secreted by the oviducts (Korschelt and Heider, 21).

Numerous minute globules adhere to the outside of the chorion, giving to it a distinctly granular appearance. That these granules are not structurally a part of the membrane is seen by the fact that when the living egg is immersed in alcohol they float away from it freely.

Viewed from the surface the yolk masses appear as a group of more or less irregular polygons (Ludwig, 24; Locy, 23; Kishinouye, 19). At first there is a slight furrowing of the periplasm, the lines of the furrows coinciding with the edges of the polygonal yolk masses. Later, however, the yolk shifts so that the edges of the masses and the furrows no longer coincide (Kishinouye, 19).

1. *The Formation of the Blastoderm.*—It is not possible for me to make a satisfactory statement concerning the earlier stages in the development of the eggs of *Epeira,* as I was unable to secure enough material of this period for a thorough study; and I am in doubt if the eggs I did obtain for the earlier stages were normal. Eggs which were killed immediately after being deposited in the cocoon, and sectioned, failed to reveal any traces of a nucleus, a condition also noted by Kishinouye in the material which he studied.

Shortly after this, however, according to Morin, Locy, and Kishinouye, a nucleus, surrounded by a mass of protoplasm, appears in the center of the egg. Kishinouye also reports the presence of a

peculiar structure, located near the nucleus, which he calls the *yolk nucleus.*

Segmentation of the egg begins, according to these authors, by the division of the centrally located nucleus into two, four, eight, etc., parts. This affects the whole structure of the egg, the yolk splitting with each division of the nucleus into a corresponding number of parts, each part containing one of the products of nuclear division (Kishinouye, 19). In this manner the yolk becomes separated into the numerous, radially arranged masses already noted, to which the name "yolk columns" has been applied.

The separation of the yolk into columns discloses a cavity in the central part of the egg. This has been regarded by some investigators as representing the segmentation cavity. It is obliterated at a later period by the incrowding of the yolk masses.

The nuclei resulting from the repeated division of the segmentation nucleus gradually pass along the lines of protoplasm which radiate outward between the yolk columns, and finally appear on the surface of the egg. They are first seen at the points where the radiating strands of protoplasm unite with the periplasm, and are equally distributed over the entire surface of the egg (Morin, 30; Kishinouye, 19).

A certain amount of protoplasm accompanies the nuclei in their outward migration (Korschelt and Heider). This unites with the periplasm. In this manner the cellular elements are formed which lay the foundation of the blastoderm. Shortly after this period, by the shifting of the yolk, the nuclei are no longer seen at the points where the protoplasmic radii and the periplasm meet, but lie more or less directly in the areas formed by the surfaces of the yolk columns. Fig. 1, *nu.*

2. *Formation of the Blastodisc.*—Following the formation of the blastoderm as a single, uniform layer of cells surrounding the egg, there is a concentration of the nuclei on one surface, namely that on which the embryo arises and which may be regarded as the ventral surface of the egg. This concentration of nuclei has been observed by most investigators; but there is a variety of opinion as to the manner in which the additional cells originate.

Both Balfour and Locy report the appearance of nuclei on the ventral surface of the egg at an earlier period than that at which they appear on the dorsal surface. Kishinouye, however, with whose observations my own accord in this respect, states that the nuclei appear simultaneously, at first, over the entire surface of the egg, and accumulate later, by division and migration, on the ventral surface.

Thus two distinct poles may be recognized in the egg of the spider; a vegetative pole, irregular in form and characterized by large, irregularly projecting masses of yolk, its surface bearing relatively few nuclei, those present being noticeably large—Fig. 2, *ylk.*—and an animal pole where the yolk is compacted to form a comparatively smooth, spherical surface, the cellular layer which covers its surface being indicated by the presence of numerous, closely crowded nuclei.

After the nuclei have migrated from the other portions of the egg to the ventral region they continue to increase rapidly in number, ultimately forming a cap of cells which covers this part of the egg. This cap is called the *blastodisc,* Fig. 2, *bld.* In the meanwhile the nuclei of those cells which have not become incorporated in the blastodisc, but have remained on the vegetative pole of the egg, increase considerably in size.

Balfour states that the endoderm is derived from cells which do not accompany the other cells in their migration to the surface, but are left behind in the yolk mass. Locy, Morin, and Kishinouye, however, failed to find any nuclei in the yolk in sections of either the blastoderm or blastodisc stages. My earliest sections, which were made with eggs whose development was somewhat in advance of the blastoderm stage of the authors mentioned, bear out the latter observations. Figs. 10, 11, and 12, *ylk.* In Figs. 15 and 16, the yolk cells—*y. c.*—are migrating from the cells of the blastodisc, from which they have been derived, into the yolk. This condition appears not only to be true of this stage in the Araneina, but was found by Patten (31) in *Neophylax,* and by Wheeler (41) in *Blatta.*

A marked feature of the blastodisc is the occurrence of a depression in about the middle of its area. (Figs. 2 and 3, *blp.* Fig. 10, *blp*) This depression is the center of an active proliferation of cells which

add to the thickness of the cellular layer in this region, and which contribute to the formation of the mesoderm. Accordingly it seems proper to regard the structure as a primitive blastopore (Korschelt and Heider).

3. *Formation of the Primary Thickening, or Primitive Cumulus.*—Following the formation of the blastodisc, there is a rapid increase in the number of cells in the region about the blastopore. Cells arising by division from the blastodisc spread out underneath that structure and form a deep layer which has not yet become differentiated into ectoderm and mesoderm.

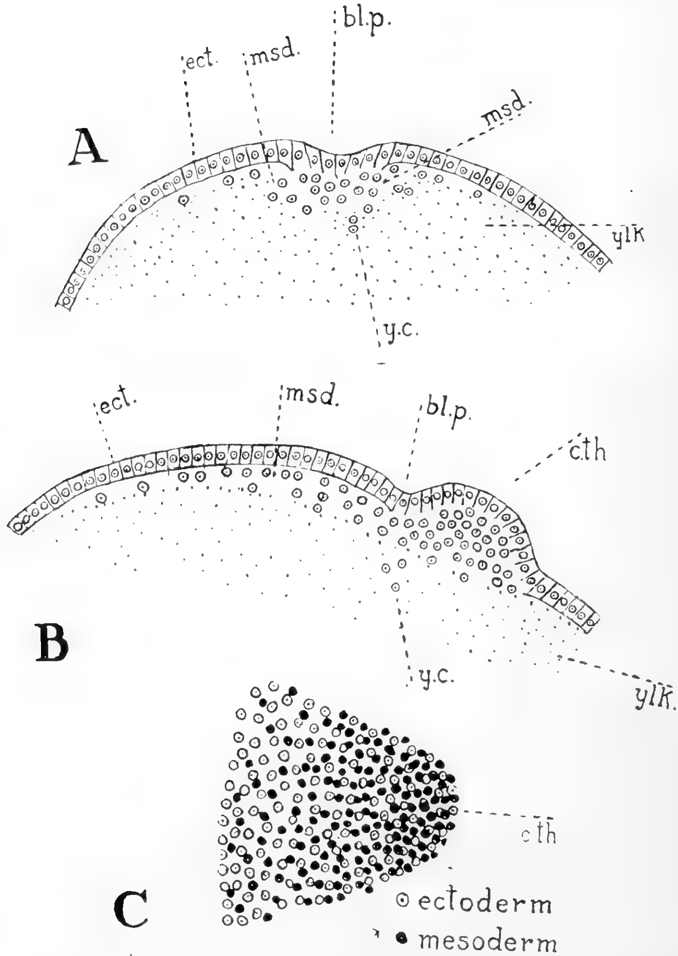
The cells sink to the greatest depth into the yolk just beneath the blastopore, which is the principal point of increase, and from which they spread out in all directions, extending even to the edge of the blastodisc.

In unstained preparations, when viewed by reflected light, the blastodisc now appears like a white cap covering the animal pole of the egg. In stained preparations it is seen to be a great accumulation of very closely packed cells. Figs. 4 and 5; *bld.*

There is some confusion among different authors as to how this accumulation of cells shall be designated. Balfour and Locy unite in calling it the "primitive cumulus," a name that was applied by Claparedé to a structure which appears later, and which has been identified as the caudal thickening. Kishinouye, to avoid the confusion arising from applying the same name to different structures, has called it the "primary thickening," which name seems best adapted to this purpose.

4. *Formation of the Caudal, or Secondary, Thickening.*—Shortly after the formation of the primary thickening, a second thickening is formed which appears as an elevation of the blastodisc near the edge of the blastopore. This elevation is caused by a rapid accumulation of cells at that point. Fig. 5, *c. th.* It is to this *structure* that Claparedé refers in speaking of the "primitive cumulus." Balfour recognized its significance and called it the "caudal thickening."

The steps in the formation of the caudal thickening may be outlined as follows. The first result of the rapid multiplication of cells in the region of the blastopore has already been described as the for-



TEXT-FIGURE 1. Illustrating the formation of the mesoderm. (Diagrammatic.)

EXPLANATION.

A. Early blastodisc stage. *bl. p.*, the blastopore-like depression in the middle of the ectoblast, *ect. msd.*, mesoderm cells, proliferating from the point just beneath the blastopore-like depression. The accumulation of these cells, and their accumulation beneath the ectoblast, forms the primitive cumulus. *y. c.*, yolk cells; certain of the proliferating cells which find their way into the yolk. *ylk.*, yolk.

B. Longitudinal section through the caudal (secondary) thickening. *bl. p.*, blastopore-like depression. *c. th.*, the caudal thickening. The point of most rapid proliferation is in the region of the blastopore. *ect.*, ectoderm. The ectoblast of the blastodisc has become a distinctive germ layer. *msd.*, cells of the growing mesodermal layer spreading out under the ectoderm *y. c.*, yolk cells.

C. Distribution of the mesodermal elements viewed from the under side. *c. th.*, caudal thickening.

mation of a cone-shaped mass that extends beneath the blastopore downward into the yolk. Text-figure 1, A. The continued formation and growth of cells in this region soon causes the blastopore to disappear, as was pointed out by Morin; the ultimate result being a pressure from underneath which causes the blastodisc to bulge outward.

The blastodisc increases in extent by the continued division of its cellular elements in radial planes. In the caudal thickening, however, many of the cells divide in tangential planes, and the new cells derived from this division are pushed forward under the blastodisc as well as downward into the yolk. Text-figure 1, B.

The caudal thickening, at this stage, has been likened to a comet, owing to the fact that the lengthening of the blastodisc, or, as it has been designated by Balfour and others, the "ventral plate," gives it the appearance of passing backward over the surface of the yolk, leaving a constantly widening trail of nuclei in its path. Text-figure 1, C.

In surface views the nuclei are seen to be arranged in the form of a triangular area covering the ventral surface of the egg. Fig. 6, *g. bd.* The base of the triangle is a broad plate from which, in a later stage, the cerebral lobes are formed. This structure is the cephalic plate. Figs. 6, 8, and 9, *c. pl.*

Sections through the early blastodisc stages show that, from the beginning, there is a rapid increase in the number of cells in the region just beneath the blastopore. This proliferation results, as has been stated before, in the formation of an undifferentiated mass of cells which projects downward into the yolk, and also in the formation of the projecting caudal thickening. From the caudal thickening new cells are formed which spread out as a broad sheet under the blastodisc (which may now be regarded as the ectoderm) thus forming the mesodermal layer.

Figs. 11, 12, 15, and 16 represent longitudinal sections made through the blastodisc at this stage. In these figures the cells of the mesoderm (*msd.*), are seen to arise from the posterior region of the ventral plate, from which point they shift forward underneath the ectoderm. These cells continue to increase in number by division

after they have left the region of the caudal thickening. In Fig. 15 some of these cells are seen to be passing into the yolk. This indicates the true origin of the so-called yolk-cells (*y.c.*). Here they increase in size by absorbing nourishment from the yolk, and form the *anlage* of the endoderm.

Thus at the time when the ventral plate of Balfour—or as I shall call it, the "germ band" (*g.bd.*)—is fully established, the three germ layers may be recognized in the embryo; the ectoderm, which consists of the cells of the blastodisc, and their immediate derivatives, which lie on the surface of the egg; the mesoderm, consisting of cells which have arisen in the region of the blastopore, and which lie immediately beneath the ectoderm; the endoderm, which is represented by a number of scattered cells that have arisen coincidently with the cells of the mesoderm, and which have migrated into the yolk.

I can find no evidence that the middle layer is augmented by the addition of cells from the outer layer (Schimkewitsch and Balfour) further than has been stated. Whatever mitotic figures have been found in the cells of the blastodisc, outside of the region of the blastopore, their position indicates that division takes place in the cells of this layer in radial planes. Fig. 14; *n.sp.* Issue must also be taken with Balfour's statement that the endoderm also contributes to the formation of the mesoderm. As later writers have shown, and as my own results indicate, the endoderm cells do not appear in the yolk until after the cells of the mesoderm have commenced to arise; the cells of both layers being derived from the same point, namely the proliferating area of the caudal thickening; the cells which pass into the yolk being thrust into that position by the conditions attending their formation, after which they take upon themselves the character of endoderm cells.

5. *The Ventral Plate.*—The germ band continues to increase in length by the rapid multiplication of its cellular elements until it covers more than two thirds of the circumference of the egg, thus forming the ventral plate. The caudal and cephalic ends of the plate ultimately come quite near one another on the dorsal surface of the egg. Fig. 9.

While this lengthening of the ventral plate is going on, some very important changes are occurring in its structure. The mesoderm cells which are, at first, spread out in a thin sheet under the ectoderm, now begin to arrange themselves in ridges which lie transversely across the band. (Fig. 8; *seg.*) These ridges, or primitive segments, are separated by furrows, each furrow being bridged by a thin sheet of ectoderm. A widening and lengthening of the cephalic plate is to be noted at this time, due to an increase in the number of its cells.

As the growth of the ventral plate continues there is a continued increase in the number of its segments. After seven or eight of these segments have been formed, small, rounded protuberances appear on the first four. These are the rudiments of the thoracic appendages. (Fig. 9; *ap.*) Locy states that the fourth thoracic appendage is the first to appear in *Agalena;* the third, second, and first, appearing in order. In *Epeira*, also, the appendages follow this order in their appearance.

After the thoracic segments are formed two appendage-bearing segments arise in the posterior region of the cephalic plate. The first of these to appear is the segment which bears the pedipalpi, the second being the segment of the cheliceræ.

When the appendages of the head and thorax have arisen, and the ventral plate has lengthened until from ten to twelve segments can be counted, it may be fairly said that the first embryonic stage has been reached.

IV. Development of the Procephalic Lobes.

Stage I. Fig. 19.—In its general development *Epeira cinerea* conforms very closely, in most points, to the development of other members of the Araneina as described by the authors already cited. Consequently, in the following study I shall confine myself to a discussion of the origin and development of the procephalic, or as they are frequently called, the cerebral, lobes and their associated sense organs.

While free use has been made of sections for the purpose of confirming results, the major part of the observations recorded were

directed to a study of the topography of the cerebral lobes in the different stages of their development. Hence reference will be made to surface views, as figured, rather than to sections.

By the time all of the appendages of the head and thorax have arisen, at least eleven segments have been established in the ventral plate. This number may be regarded as being characteristic of this stage. The plate also has lengthened until it has nearly encircled the yolk. (Fig. 9.)

The nuclei of the cells composing the embryonic tissues are quite small, and are closely crowded together. The nuclei which lie outside the embryonic tissues, and which are scattered irregularly over the surface of the yolk, are conspicuously larger.

The ventral plate is divided into two lateral halves by a broad median furrow (*l. fr.*) which begins at a point not far from the anterior margin of the cephalic plate, and extends posteriorly along the mid-ventral line to the margin of the caudal lobe. This furrow is bridged by a thin layer of ectoderm which connects the lateral halves of the embryo. The lateral halves of the ventral plate are composed of clearly defined segments.

Two segments lie anterior to those which bear the thoracic appendages. The posterior of these is associated with the rudimentary pedipalpi; the other bearing the rudiments of the cheliceræ. In this stage each of the segments of the thorax bears a pair of rudimentary appendages. The remaining segments belong to the abdomen, and increase in number as the embryo continues to lengthen. No appendages appear, however, on the abdominal segments during this stage.

That portion of the cephalic plate which lies anterior to the cheliceral segment does not present any marked differentiation at this time, except that the broad, lateral areas, where the optic ganglia (*o. gl.*) appear at a later period, are considerably thickened. In transverse sections of the cephalic plate these lateral portions are seen to consist of ectoderm cells which lie several layers deep. A small groove appears, on each side of the head, in the posterior region of this thickened area (*lat. gr.*). These are the beginnings of the invaginations which form the lateral optic vesicles.

In this stage we find that the middle region of the cephalic plate, which lies close to the anterior margin, has become slightly raised by the thickening of the ectoderm. This can be readily determined by manipulating the egg with needles so that the structure can be clearly seen by reflected light. The prominence formed by this elevation of the ectoderm is the foundation of the future rostrum, or upper lip (*ros.*)

Stage II. Fig. 20.—The second embryonic stage is marked by a slight advance in the development of the cephalic plate. The number of body segments may have increased, but there is no fixed number which can be regarded as characteristic of this period. The rudiments of the thoracic appendages have also become more prominent. Of the head appendages, the pedipalps especially have lengthened, and now show a tendency to bend toward the median line, a position which is characteristically maintained by all the appendages except the cheliceræ up to the time of hatching.

A characteristic feature of this stage, to which attention should be called, is the conspicuous furrow which separates the cheliceral segment from the pre-cheliceral part of the cephalic plate. It thus becomes evident that all of the appendage-bearing segments belong to the same series and can not be distinguished from one another in the earlier stages as the use of the terms "thoracic" and "cephalic" appendages would seem to indicate. The union of the first two appendage-bearing segments with those portions of the embryonic plate which give rise to the cerebral structures is a distinctly later event.

The development of the lateral areas of the cephalic plate has continued until the ectoderm is deeper in this region than in any other portion of the plate. The appearance of the lateral tracts in surface views as a considerably darker area is due to the greater thickness of the ectoderm, and the closely crowded nuclei of its cells (*o. gl.*). The rudiment of the rostrum shows a slight advance by its more marked separation from the adjacent parts (*ros.*).

Stage III. Fig. 21.—In this stage a distinct condition of advance is to be recognized in the development of the head region. The lateral, or optic, area is more certainly differentiated from the medially

located cerebral area, the entire cephalic plate being raised into three transverse ridges which are particularly prominent in the optic region where they form the beginning of the optic ganglia (*o. gl.*).

Between these rudimentary optic ganglia and the longitudinal furrow on each side of the cephalic plate, the transverse ridges present less conspicuous elevations, which form, in a similar manner, the foundations of the cerebral ganglia (*c. gl.*). Thus we find in this stage that the pre-cheliceral portion of the cephalic plate bears three transverse segments, each segment consisting of a cerebral and an optic portion, a condition which Patten found in both *Acilius* and *Buthus* (No. 35).

The lateral invaginations, or grooves (*lat. gr.*), now become deeper, folding inward until the result is the formation of a pit, the blind end of which lies toward the median line underneath the optic ganglia.

At this time a thickened rim (*o. pl.*) appears on the outer margin of the optic area on each side of the head. The sensory character of this structure was first established by Patten (33 and 35) who first observed it in *Acilius,* calling it the optic plate on account of its relation to the future formation of the eyes. Later the same author identified a similar structure in *Buthus* and *Mygale.*

Thus it appears that the cephalic plate of *Epeira,* anterior to the cheliceral segment, from which it is still separated by a conspicuous furrow on each side of the head, is composed of three distinctly marked, longitudinal areas—a median area which constitutes the foundation of the cerebral ganglia; a marginal area, forming the rudiment of the optic ganglia; and a thickened marginal rim, the optic plate, which forms, at a later period, the retinal portion of the anterior median eyes.

The broad elevation of the ectoderm which has been already recognized as the rudiment of the rostrum, has now formed two separate prominences (*ros.*). These prominences have shifted downward from the anterior margin of the head and lie between the cerebral ganglia. A slight depression of the ectoderm, or ectodermal pit, between the two rostral elevations indicates the beginning of the stomodæum (*st.*).

In sections these elevations are seen to be lined with mesoderm, and enclose a portion of the cœlomic cavity, a fact which indicates their appendage-like character. This double origin of the rostrum was shown by Schimkewitsch, who also pointed out the fact that, as indicated by their structure, these prominences represent a pair of cephalic appendages.

About this time numerous pits appear in the ganglionic areas of the cephalic plate (*n. b.*). Each pit arises as an independent structure, and is surrounded by a well-marked circle of nuclei. In surface views they appear to be like small, light cells, arranged with considerable regularity in the ectoderm. Viewed superficially they appear to contain no nuclei; but on close inspection nuclei may be discerned lying below the surface of the depression, thus indicating the position of the cells of which it is composed.

In sections these pits are seen to be surrounded by cells having a considerable regularity of arrangement. The hollow of the pit is filled with a clear substance, apparently secreted from the surrounding cells.

At first sight these pits bear a close resemblance to the structures which Wheeler found in *Xiphidium*, to which he applied the name of "neuroblasts." The neuroblasts of Wheeler are described as being "numerous, large, light cells in the surface of the ectoderm" from which the elements entering into the structure of the sense organs are derived by proliferation. In *Epeira*, however, the pits are, as has been indicated, more or less regularly arranged depressions of the ectoderm; the cells surrounding each depression being grouped together in a manner which strongly suggests the way the optic cells are arranged in a simple ocellus.

Patten has expressed the belief that these pits represent primitive sense organs, rather than being, like the neuroblasts of Wheeler, the points of origin for numerous sensory elements. Patten has shown that these primitive sense organs form at a later period, by proliferation and transformation, special groups of ganglion cells. Moreover, since, as the same author has clearly shown, the minute structure of these pits is the same as that of true peripheral sense organs which occur at the base of the legs in scorpions, one can scarcely avoid adopting his point of view.

The sensory pits arise first in the ganglionic areas of the cephalic plate. Later, however, they appear in the thickened median portions, or neuromeres, of the thoracic and abdominal segments, as well as in other parts of the embryo which are destined to enter into the formation of the sense organs.

The appearance of these primitive sensory pits in the cephalic plate, in the rudiment of the nerve cord, and in other sensory structures of *Epeira,* strongly favors the view that the central nervous system arises, phylogenetically, not by the multiplication of simple neural elements, but by the transformation and aggregation of *primitive sense organs* into the parts from which the nervous system is derived.

Stage IV. Fig. 23.—The first change to be noted in this stage is the increase in the size of the appendages which have become longer and thicker in a marked degree, the pedipalps having developed a distinct coxal portion. Slight transverse constrictions appear in the pedipalps and in the thoracic appendages, which are indicative of future segmentation.

In the body of the embryo the wide separation of the lateral plates has caused the thoracic appendages to be further removed from the median line than is the case with the appendages of the head. Small knob-like projections appear on the second, third, fourth, and fifth abdominal segments. These are rudimentary abdominal appendages. There has been some discussion concerning the appearance of a first, limbless abdominal segment in the Araneina. Schimkewitsch (38) and Bruce (5) report a segment which fails to bear any trace of an appendage, appearing between the last thoracic segment and the first limb-bearing segment of the abdomen. Kishinouye (19) also found that appendages were wanting on the first abdominal segment of the spiders which he studied.

On the other hand, Balfour (1) and Locy (23) show in their drawings appendages on the first segment of the abdomen. Korschelt and Heider in the "Lehrbuch der vergleichenden Entwicklungsgeschichte" figure an Araneid embryo which has a pair of appendages on the first abdominal segment, as well as on the others, making five pairs of abdominal appendages in all. The accuracy of this

figure appears to me doubtful, since no other investigators have reported more than four pairs in all. In *Epeira* the first abdominal segment is very distinct, but never at any time does the trace of an appendage appear upon it.

It is possible that in some species of the Araneina the first abdominal segment is not very clearly defined, and consequently has been overlooked by those who have failed to report it as a limbless segment. On the other hand it may have been obliterated by the strong tendency manifested by the most anterior of the abdominal segments to shift forward into the cephalo-thoracic region.

The yolk, in this stage, bulges out between the lateral plates, and is covered by a thin layer of ectoderm. The lateral plates are distinctly segmented, the segments extending to the dorsal margins of the plates. The neuromeres which form the foundation of the nerve cord are well formed on the median margins of the plates.

Several important changes have taken place in the cephalic plate. The first of these is the formation of the semi-circular, or anterior, groove (*ant. gr.*). This groove appears as a crescentic invagination near the anterior margin of the head, and extends around the sides to a point a little in front of the lateral grooves. Longitudinal sections show that the groove is formed by the infolding of the ectoderm in this region, shallow at first, but becoming gradually deeper and deeper as the development of the embryo progresses. The lateral grooves, at the same time, have deepened considerably and form conspicuous pits on the lateral margins of the head (*lat. gr.*).

Each lobe of the pre-cheliceral portion of the cephalic plate consists of three segments, the first lying in the region of the anterior groove, not far from the margin of the head, the third being in close proximity to the cheliceral segment (cgl^3 and *chl. seg.*). In some instances, in embryos of this stage, the cheliceral and third cephalic segments lie directly against one another.

The thickened portions of each of these segments lying toward the median line (*c. gl.*) forms a neuromere-like structure which, as has already been pointed out, forms the foundation of a cerebral ganglion. The series of cerebral ganglia, in each lobe of the cephalic plate, is continuous with the chain of neuromeres of the thoracic and abdom-

inal segments, from which the nerve cord is derived. Thus the cerebral ganglia are seen to maintain the same relation to the precheliceral segments that the neuromeres which form the rudiment of the nerve cord hold to the thoracic and abdominal segments, and may be regarded as being serially homologous with them.

The first, second, and third optic ganglia now appear as thickened areas which lie between the cerebral ganglia and the outer margins of the lobes. The optic plate(*o. pl.*), or thickened margin of the optic ganglia, consists of a continuous band of thickened ectoderm bordering the lateral edges of the cephalic plate.

Stage V. Fig. 24.—The dorsal flexure reaches its extreme limit during the preceding stage. After this period a remarkable change occurs, and, instead of the cephalic and caudal ends of the embryo approaching one another on the dorsal side, the embryo is gradually bent in the other direction so that the two ends approach each other on the ventral surface.

According to Balfour and Locy, with whom Kishinouye is in substantial agreement, this change of flexure from the dorsal to the ventral direction is due to the increasing growth of the dorsal surface and the shortening of the germ bands. Both of the authors referred to fail to mention what is, in their opinion, the cause of this shortening of the lateral plates of the embryo.

Morin maintains that the change is due to the shifting of the yolk from the ventral to a more dorsal position. But it is to be observed in *Epeira* that the yolk never bulges ventrally in a more prominent manner than during the first stages of the reversion period, a condition which the greater extension of the lateral plates over the dorsal surface of the egg is well calculated to produce.

It seems probable that Balfour and Locy have given a correct answer to this problem in so far as we are to look for the cause of this change, not in the shifting of the yolk, but in the growth of the embryo itself. Since the position of the yolk is purely relative, any change in the growth of the lateral plates would result in a change in its position. The growth of the lateral plates continues dorsalwards until they meet in the mid-dorsal line where they enclose a portion of the original cœlom which becomes the cavity of the heart.

After the union of the two halves of the dorsum their continued growth produces a pressure upon the most posterior of the abdominal neuromeres which causes them to shift anteriorly; a process which results in the shortening of the entire ventral surface of the embryo, the shifting neuromeres becoming associated with segments anterior to those with which they originated.

The results of the forward shifting of the neural elements are also evident in the cephalic plate. The cheliceral segment is among the first to respond to this crowding of the neuromeres in a forward direction, so that it becomes closely pressed against the segment in front, thus entirely obliterating the furrow which separated these two segments in the preceding stages.

The anterior groove is not greatly influenced by this pressure from the posterior direction in this stage, the lips of the invagination being quite widely separated. The optic and cerebral ganglia are quite distinct, and are easily distinguished in surface views. The examination of these elevations of the ectoderm in the cephalic region is facilitated by rolling the egg from side to side, a process which brings the contours into more perfect relief.

The rostrum (*ros.*) which had shifted, in the preceding stage, from its first position near the anterior margin of the head, has moved still farther in the posterior direction.

One of the most marked advances which the embryo has made in this stage, is the beginning of the structure which Patten describes for the scorpion, and to which he has given the name "cephalic fold" or "hood." As the growth of the cephalic lobes progresses, their margins, which consist of the thickened limb of ectoderm, the optic plate, is raised slightly and turned in the medio-posterior direction. The fold (*o. f.*) thus formed continues to advance in this direction as the development of the embryo continues, bearing the inverted optic plate on its margin. In this stage the fold progresses until it has come to lie above the opening of the anterior groove, and the more anterior portions of the cephalic lobes.

Stage VI. Fig. 26.—The parts of the cephalic lobes which, in the preceding stages, have been quite distinct, are now beginning to lose their identity by uniting with one another. The cerebral ganglia are

fused into a large lobe, which has become considerably thickened by the continued increase in the number of its cells. The optic ganglia have united to form a considerably broader plate, which occupies the greater part of the superficial area of the cephalic lobes.

The sensory pits in the optic lobes have become more obscure, while those of the cerebral ganglia are separated from one another, in a measure, by the breaking up of the ganglia into blocks, each block containing a sensory pit. This separation is brought about in such a way that the cerebral ganglia seem, in surface views, to have been fractured. The significance of this condition is not clear.

The rostrum has, in this stage, lost all evidence of its double origin; and is a broad, plate-like structure which entirely covers the stomodæum. The different elements of the cephalic plate have shifted their positions in such a manner that the rostrum lies almost between the two halves of the cheliceral segment.

The anterior grooves (*ant. gr.*) have come together in the median line, forming a continuous, semi-circular depression which extends around the anterior margin of the cephalic plate. This groove has deepened considerably, and will be referred to as the anterior optic invagination on account of the intimate connection which exists between it and the innervation of the median eyes. The lateral grooves (*lat. gr.*) will be referred to as the lateral optic invaginations, holding the same relation to the lateral eyes that the anterior optic invagination holds to the median eyes. The anterior and lateral optic invaginations form important parts of the optic ganglia.

There is a shallow depression which appears between the lateral and anterior invaginations, on each side of the head (*m. o. p.*). That this pit may be regarded as the vestige of a more elaborate structure than it now is, probably a third invagination associated with the formation of the optic lobes, appears evident from the fact that it occurs constantly in the cephalic plate of embryos of this stage.

The shifting of the neuromeres of the nerve cord, and the related shortening of the ventral surface of the embryo, brings a certain amount of pressure to bear on the cerebral lobes which causes them to be pressed over the anterior optic invagination, thus forcing the lips of that infolding together. The edge of the cephalic fold (*o. f.*)

also advances in the medio-posterior direction, its increased growth making its character somewhat more apparent.

The pedilaps and cheliceræ have continued to enlarge; a mandibular segment being formed by the growth of the basal joint of the pedipalps in the median direction. This segment is separated from the main portion of the pedipalp by a deep constriction at the point where the appendage and the coxa are united (*md. pdp.*).

It is to be observed, in all stages, that the development of the cephalic plate does not, in every case, keep pace with the progress made by the remaining parts of the embryo, the body frequently showing a state of development which is in advance of what may be regarded as the normal condition of the cephalic plate. Occasionally embryos are found in which the two halves of the brain do not keep pace with one another. Continued growth, in most cases, equalizes these irregularities; although, in rare cases, the divergence becomes so great that an abnormal structure of the embryo is the result.

The most frequent of these abnormalities to be met with is the double embryo, in which the anterior portion of the germ band grows in two directions, two cephalic plates being formed. A single caudal plate serves for the two embryonic bands which unite in the thoracic region.

Stage VII. Fig. 27.—From this point on the coalescence of the ganglionic elements of the cerebral lobes proceeds with great rapidity and regularity. The lips of the anterior optic invagination have closed so that the groove can be made out with difficulty in surface views. The closure is apparently due, as are nearly all the important changes which are taking place in the form of the cephalic plate, to the forward migration, and to the general concrescence, of the neuromeres. This closure is more apparent in the lateral parts of the invagination, being less complete in the median portion of the lobes.

The anterior optic invagination has increased in depth to the extent that it now forms a large vesicle which has come to lie underneath the anterior margin of the cerebral lobes. The anterior, or dorsal, lip of this vesicle is directly connected with the optic plate

by means of a sheet of ectoderm which forms the inner layer of the cephalic fold. (Text-figure 3, *ret.*) The posterior lip of the vesicle is directly continuous with the cephalic lobes.

The openings of the lateral invaginations are no longer to be seen in surface views. Sections show that vesicles have been formed by these invaginations which lie beneath the lateral margins of the optic lobes, each vesicle possessing a lumen of considerable size.

The cerebral lobes have continued to increase in thickness, partly by a continued increase in the number of their cells, and partly by the crowding of the neural elements of the lobes into a more compact mass.

The shifting of the neuromeres has placed the two halves of the cheliceral segment laterally to the stomodæum which is now a deep invagination of the ectoderm covered by a flat, triangular rostrum. The lateral eyes first appear as thickenings of the ectoderm (*l. e.*) at a point on the margin of the cephalic plate somewhat posterior to the position of the lateral vesicles.

Immediately below the rostrum a small depression is to be seen in the ectoderm which bridges the longitudinal furrow (*c. p.*). Patten found a chain of such pits between the segmental neuromeres of the scorpion, and related them to the "mittelstrang" of Hatscheck. According to Patten, this chain of pits gives rise to the bothroidal cord. In *Epeira,* however, I have been unable to discover more than the single pit.

A transverse section made through the region of the stomodæum shows that structure to be flanked on either side by a lateral ganglion of considerable size. These ganglia arose in connection with the cheliceral segment, and they are now connected by a strand of nerve tissue which passes from one to the other beneath the stomodæum (*s. s. c.*). These ganglia, at a later period, become fused with the main portion of the brain, the connecting strands of nerve tissue forming the sub-stomodæal connectives which are present in the adult brain of the spider (St. Remy, 39).

Stage VIII. Fig. 28.—This stage shows many features of advance in the morphology of the cephalic plate. The cephalic fold has grown until it covers a little more than the anterior third of the brain (*o. f.*).

In following the development of this fold we find that it arises, as has been previously pointed out, as a thickening of the edge of the plate which becomes turned upward and backward over the lobes. Its continued growth causes it to advance toward the median line, and in a posterior direction over the lobes, the thickened portion remaining on the edge of the fold. The fold may consequently be described as an ectodermal mantle consisting of two parts, an inner and an outer. The outer portion of the mantle consists of a single sheet of cells which extends dorsalwards and becomes continuous with the ectoderm of the dorsum. The inner part is several layers of cells thick and lies close to the cerebral lobes, being continuous with the dorsal lip of the anterior optic invagination. On account of the infolding of the ectoderm to form the inner layer of this structure, the thickened optic plates come to lie in an inverted position near the edges of the fold, and form the retinas of the anterior median eyes.

The pressure of the neuromeres which have crowded into the region of the head and thorax has brought about a still greater concentration of the neural elements. In the head the various ganglia are compacted into a single mass of nerve tissue in which the individual parts can be distinguished with difficulty. The anterior optic invagination has also increased in size, its walls having become so much thicker that its lumen is completely obliterated and the entire structure has come to lie, to a greater extent, underneath the cerebral lobes.

Another marked feature of this stage is the lessening of the superficial area of the head. During the first stages in the growth of the embryo, the cephalon lies as a broad plate on the surface of the yolk. As its development progresses, and the fusion of originally distinct parts occurs, accompanied by the sinking of certain of the superficial portions below the surface, a reduction of the superficial area of the head ensues. This process is particularly evident in the narrowing of the anterior portion of the head.

Stage IX. Fig. 30.—In surface views of this stage the optic fold is seen to cover the anterior half of the optic lobes. Its posterior margin forms an inverted V, the arms of which diverge considerably. The more anterior portions of the fold have met in the median line.

The fold, together with the optic and cerebral lobes, form a true cerebral vesicle; the roof of the vesicle consisting of the fold, itself, the floor being made up of the ganglionic portions of the cephalic plate. The lumen of this vesicle extends dorsalwards, its opening being in the ventral direction.

A considerable change has also taken place in the position of the ganglia of the head. As has already been indicated, the concentration of the neural elements, owing to the massing of the neuromeres in the head region, has resulted in pushing some of the ganglia below the surface. As a result of this two parts of the brain may now be distinguished according to the level in which they lie; first, a superficial part which forms the optic portion of the brain, and, second, the large optic vesicle which lies beneath the anterior margin of the cerebral lobes (the *organ stratific* of St. Remy), together with the lateral optic vesicles, and a median portion, the cerebral ganglia which have, by this time, come to be overlaid by the optic ganglia.

The cephalon is divided into two distinct parts by the deepening of a constriction which appears at a somewhat earlier period (cf. Fig. 29) and separates the region in which the anterior optic vesicle lies from the more posterior portions of the cephalic plate (*m. f.*).

The cheliceræ have been pushed dorsalwards until they lie above the rostrum, which they partially conceal. The segment bearing the pedipalps now lies immediately beneath the stomodæum.

The neuromeres of the thoracic segments appear as oblong masses of nerve tissue, each segment being separated from the one anterior to it by a small, but distinct, furrow. In this the Araneina repeats the condition which Patten (35) found in the scorpion, except that the neuromeres, themselves, fail to show any indication of a division into two parts.

At this stage in the development of the embryo the *mittelstrang* appears as a shallow, longitudinal furrow between the two halves of the nerve cord (*ec. f.*). This furrow is not extensive. Its point of origin, in the region of the stomodæum, is hidden by the lower margin of the rostrum. It ends in the structure which was noted in the previous stage as the "ectodermal pit" (Figs. 28, 29, and 30, *e. p.*), which is now located between the neuromeres of the first thoracic segment.

Stage X. Fig. 31.—Progress toward the adult condition is well marked in the embryos which have been selected to represent this stage. The brain is seen to consist of two distinct parts, the procephalon, or supra-œsophageal (Fig. 34, *br.*) mass, and the post-cephalon, or sub-œsophageal mass (Fig. 34, *s. o. g.* 1-12). The lobes of the pro-cephalon do not become so perfectly fused that they lose their identity until a little later. The ganglia of the post-cephalon, although pressed closely together, are never fused.

Practically the entire area of the cephalic plate is covered by the optic fold, which now extends to, and partially covers, the base of the cheliceræ. The parts of the fold which bear the rudiments of the anterior median eyes have come together in the median line. The ectodermal thickenings which form the rudiments of the lateral eyes (*l. e.*) are located on the lateral margins of the head, not far from the bases of the cheliceræ.

The ganglia of the thoracic segments have crowded forward until they are packed closely together just beneath the stomodæum; the remainder of the thorax beneath the œsophagus being filled with ganglia consisting of the neuromeres of those abdominal segments which have shifted forward into this position.

The formation of the *mittelstrang* is completed by having the lips of the ectodermal furrow unite to form a tube which lies just beneath the ectoderm between the neuromeres of the first abdominal segment. This tube ends, posteriorly, in the region in which the ectodermal pit was located. In the method of their formation the furrow and the pit resemble very closely the infoldings which develop, in part, into the "limatochord," and, in part, into the intra-ganglionic commissures which Patten (35) mentions in connection with his description of *Buthus.*

In front of the antero-lateral margins of the head the ectoderm is raised into a slight fold (*a. f.*). This fold is probably to be compared with the so-called amniotic fold of insects.

Large cells with peripherally located nuclei, which are present near the margins of the embryonic plate in nearly all of the preceding stages, appear, in this stage, to be gradually sinking into the yolk. The precise nature of these cells is doubtful. They have been regarded

variously as representing the *anlage* of the endoderm, and as being the cells from which the blood corpuscles are derived. That they are not blood cells is clearly evident from the fact that after the formation of the heart by the union of the two walls of the dorsum, no cells of this sort are to be found in its lumen. At the same time they are abundant at the posterior end of the stomodæum, and in the region of the proctodæum and stercoral pocket, as well as being noticeably abundant in the region where the genital organs of the spider are formed.

Stage XI. Fig. 32.—This is the earliest period in the development of the spider in which the various parts of the brain have assumed approximately the position they will occupy throughout the life of the adult. In external form the young spider closely resembles the adult, although no pigment has appeared in either the eyes or the skin.

The internal organization of the embryo is, however, very incomplete. The lung-books have arisen; the spinning glands have formed and are nearly functional. But the mid-gut is lacking (Fig. 34, *oes.*), the abdomen being filled with a mass of modified yolk which is traversed by rudimentary vessels, and by septæ, springing from the walls of the heart.

The anterior median eyes have advanced considerably in their development. The corneal layer of the eyes is directly continuous with the ectoderm which covers the outer portion of the head; the infolded portion of the eyes bears the retina and, turning again, posteriorly, forms a third, or post-retinal layer. (Fig. 33, *m. e.*)

The posterior median eyes and the lateral eyes are also considerably advanced. Following Bertkau (4) these may be called accessory eyes *(nebenaugen)*, the anterior median eyes being called by this author the principal eyes *(hauptaugen)*.

The accessory eyes do not arise in relation to any special infolding after the manner of the principal eyes. They first appear as thickenings of the ectoderm in the region of the optic plate, and are situated a little in advance of the bases of the cheliceræ. As the optic fold advances the accessory eyes become located on its outer portion, the thickened area, in the case of each eye, forming the bottom of a

cup-shaped depression, and becomes the retina of the eye (*p. m. e.* and *l. e.*).

The larger and more superficial regions of the procephalon consist of the optic ganglia which overlie the anterior and lateral optic vesicles, as well as the ganglia of the cerebral lobes.

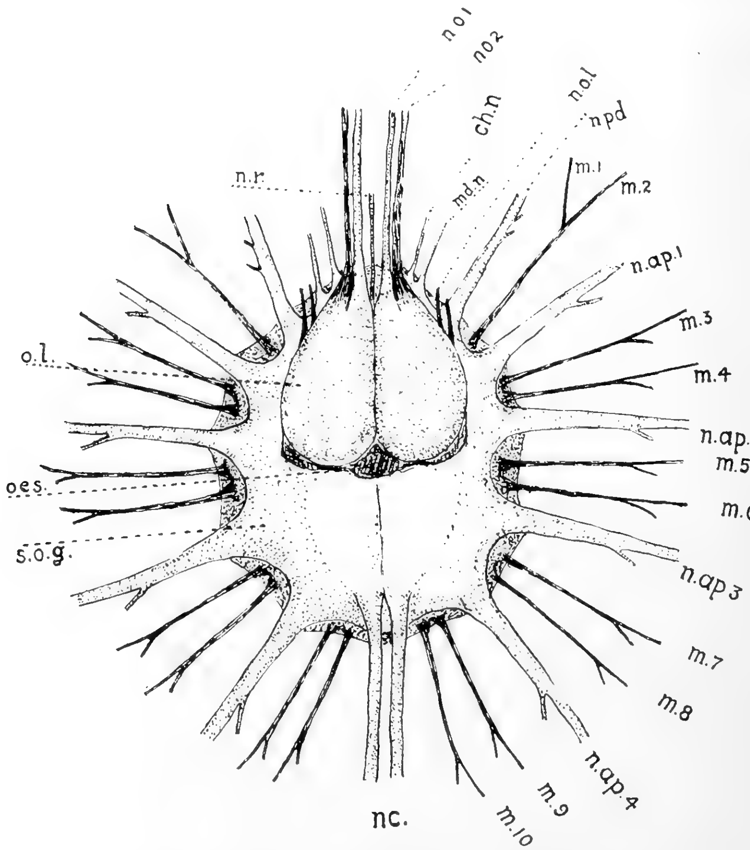
V. Formation of the Adult Brain.

The procephalic portion of the brain of *Epeira* is a structure of considerable complexity, consisting of two lateral, pear-shaped lobes which are separated by a deep longitudinal furrow. (Text-fig. 2; *o. l.*). As we have seen, however, in following the development of the brain, each of these lobes consists of optic ganglia and optic vesicles, which constitute the greater part of their superficial area (Fig. 27; *opt. g.*). The lobes formed by the cerebral ganglia lie underneath the optic ganglia (Fig. 27; *c. gl.*). The short, thick, column-like connectives on either side of the œsophagus, which unite the procephalic and postcephalic portions of the brain, represent the contribution of the cheliceral segment to the cerebrum of the spider.

Below the œsophagus is a mass of ganglia consisting of neuromeres, some of which belonged originally to the abdominal segments (Fig. 34; *s. o. g.* 1-12). Their position in the head region is one of the results of the reversion of the embryo, which caused them to be pushed forward into that place. These ganglia are imperfectly fused, even in the adult, a fact which facilitates their determination. The first four of these sub-œsophageal ganglia arose in connection with the thoracic segments, the rest having been crowded forward into the thoracic region from the places where they originated in connection with the segments of the abdomen.

These observations bring the brain of *Epeira* into close accord with the researches of St. Remy and Patten. St. Remy speaks of the procephalic portion of the brain as consisting of a rostro-mandibular part (cheliceral ganglia), and a much larger optic ganglion which consists of the principal portion of the supra-œsophageal mass. In the optic ganglion he found three distinct divisions, the first of these he calls the *optic lobe.* The second he calls the *cerebral lobe.* Behind these lies the unpaired *organ stratifie* which, together with the optic ganglia, comprises what I have called the optic lobe.

The conditions which obtain in the development and structure of the brain of the Epeiroid spiders fulfil the conditions of this descrip-



Text-Figure 2. Brain of the Epeiroid spider, *Argiope riparia*, viewed from above. *n. o*1—*n. o*2, optic nerves supplying the median eyes. *ch. n.*, cheliceral nerve. *md. n.*, mandibular nerve. *n. pd.*, pedipalpal nerve. *m*1—*m*10, segmental nerves supplying the muscles and the body wall. *n. ap*1—*n. ap*4, nerves to the thoracic appendages. *n. c.*, nerve cord. *o. l.*, optic lobes (supra-œsophageal brain mass). *œs.*, œsophagus. *s. o. g.*, sub-œsophageal ganglia.

tion perfectly. The commissures which St. Remy found uniting the lateral portions of the procephalic lobes, are present in the earlier stages of the brain as the supra- and sub-œsophageal commissures.

The procephalic lobes lie not only above, but a little in front of, the postcephalic ganglia (text-fig. 2). A large, almost tube-like, pair of nerves, which are more dorsally placed than the others, extends from the brain to the anterior median eyes. The nerves supplying the posterior median eyes arise from the surface of the brain near those which supply the anterior median eyes, and follow almost parallel with them, though occupying a somewhat lower level. The nerves which supply the lateral eyes arise from a more lateral position on the side of the brain, in conjunction with the lateral optic vesicles.

The remaining nerves are all given off from lower levels. They consist of a fine strand of nerve fibers which arises in the median line, between the optic lobes, and passes to the rostrum; and the cheliceral nerves, which arise from the upper part of the connectives, or ganglia of the cheliceral segment.

The mandibular and pedipalpal nerves arise from different levels of the pedipalpal segment. The remaining portions of the postcephalic ganglia give off, on each side, the four large nerves which pass to the appendages. The nerve cord consists of two large, tubular nerves which arise from the dorsal surface of this portion of the brain.

Between the appendicular nerves are four pairs of fine, thread-like, accessory, or segmental, nerves on each side of the brain. These nerves supply the thoracic muscles and the body wall. The nerves comprising the first segmental pair are fused, although they separate into two branches a short distance from their point of origin.

The paired condition of the segmental nerves does not appear in the scorpion according to McClendon (26), although Patten, who studied the same species, seems to have had no difficulty in making them out.

VI. The Eyes of Epeira.

No problem in Arthropod embryology has attracted more attention, or yielded more interesting results, than the study of the development and relationships of the eyes of Arachnids. The most important of these observations are to be found in papers by Bertkau (4), Schimkewitsch (28), Locy (23), Mark (25), and Patten (33, 35).

Schimkewitsch's paper deals accurately with the general morphology of the eyes, but fails to relate them properly with the optic ganglia. His statement that the optic nerves are derived from the *cephalic ganglia* is somewhat ambiguous, although he probably refers to the procephalic lobes.

One of the most important works relating to the subject is the paper by Locy on the development of *Agalena nævia.* Although he failed to understand the significance of the fold which forms the roof of what he calls the "optic vesicle" (the cerebral vesicle), he was the first to correctly describe the morphology of the anterior median eyes.

His conclusions concerning the mode of origin of the posterior median, and lateral, eyes need revision, as has been pointed out by Kishinouye. Locy holds that the accessory eyes arise in association with ectodermal infoldings in a way somewhat similar to that in which the principal eyes are formed. The ectodermal invaginations with which the lateral eyes are associated in their formation, however, enter into the formation of the optic lobes, and form the point of origin from which the optic nerves, supplying the lateral eyes, arise. They do not, themselves, give rise to the eyes.

He calls attention to a very important point, that is, the inversion of the retinal elements which is brought about by the infolding of the optic fold, thus explaining how it occurs that "the way in which the light traverses the median anterior eyes of spiders is similar to the method by which light reaches the percipient elements in the retina of the vertebrate eye."

Another important paper which deals with the subject is that by Kishinouye. He recognizes the fact that the elements which enter into the formation of the anterior median eyes are originally located on the *posterior margins of the covering of the cerebral vesicle;* but he fails to note that these optic elements are brought into this position from the lateral margins of the cephalic plate. He finds, following Locy, that the inversion of the retinal elements of the median eyes is due to the "processes by which the eyes are formed."

Kishinouye also states concerning the origin of the optic nerves, that they arise as elongations of the retinal cells, forming fibers which

become secondarily connected with the optic ganglia, this connection taking place after the eyes are fully formed.

His observations on the manner on which the accessory eyes are formed appear to be confirmed by my own observations of the way in which they arise in *Epeira,* and probably hold true for all spiders. He finds that these eyes do not arise by a process of infolding, but as simple depressions of the ectoderm, which is thickened in the optic area to form the retina. The walls of this depression grow inward so that they finally cover the retinal portion of the eye.

Patten was the first to point out that the eyes of Arachnids consist of three layers, an outer, or corneal layer, a middle, or retinal layer, and an inner, or post-retinal layer. He also called attention to the fact that, not only in the manner in which light traverses the retinal elements, but in their mode of origin as well, the anterior median eyes of Arachnids bear a close resemblance to the pineal eyes of vertebrates, with which they are compared (35).

One of the most important contributions which this author has made to our knowledge of the morphology of the Arachnid eye, is his discovery that the optic elements do not arise originally in the median position, but first appear on the margins of the cephalic plate. Their ultimate median position is brought about by the reflexing of the margins of the cephalic plate over the optic ganglia and the cerebral lobes.

Mark's paper (25) is an important contribution to the discussion of the way in which the mode of the formation of the Arachnid eye bears on the question of the morphology of the eyes of Arthropods in general, following for his account of the Arachnid eye the work of Locy on *Agalena.*

The Anterior Median Eyes of Epeira.—The anterior median eyes originate as sensory thickenings of the antero-lateral margins of the cephalic plate. (Figs. 19-29; *o. gl.*). As the development of the cephalic lobes progresses, that portion of the margin upon which the optic plate is located is turned upward and backward in such a manner that a fold is formed which covers the anterior portions of the brain (Figs. 27-31; *o. f.*). The edge of this fold advances over the cephalic plate in the medio-posterior direction, the two halves of the fold meeting, as they progress downward, in the median line.

By means of this process the optic thickening comes to be turned toward the ganglia of the brain; the retinal elements being literally turned upside down, which accounts for their inverted position in the adult eye.

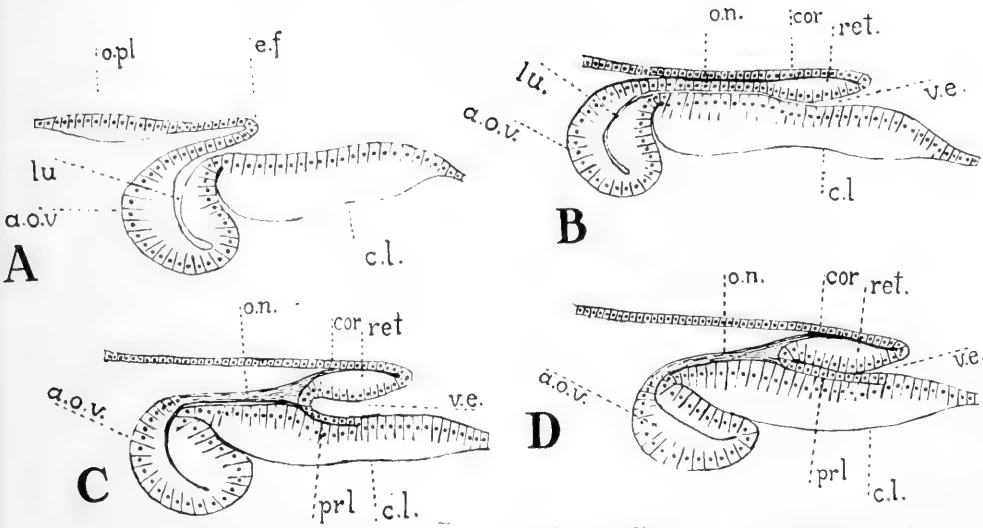
The result of this growth of the fold is the formation of a sac-like vesicle which is open in the posterior direction. The fold, itself, forms the roof of this vesicle, while the floor consists of the optic and cerebral ganglia of the brain (text-fig. 3, B; *v. c.*). The lumen of this sac is continuous with that of the anterior optic vesicle. (Text-fig. 3, B; *lu.*).

As the growth of the cerebral lobes continues, they overlie more completely the vesicle formed by the anterior optic invagination, the lumen of which is finally closed by the thickening of its walls, and by the pressure which is brought to bear upon it from above. (Text-fig. 3, C and D; *a. o. v.*).

The formation of the nerves supplying the anterior median eyes has been accounted for in two ways; first, as outgrowths from the optic tracts of the brain, and, second, as elongations of the retinal cells which become secondarily attached to the optic lobes.

There is a third way which a careful examination of my material has led me to believe may account for the formation of the optic nerves. As the growth of the optic fold progresses, its inner layer, connecting the retinal thickening with the dorsal lip of the optic vesicle, comes to lie close upon the optic lobes, and finally unites with them (text-fig. 3, B; *o. n.*). The lower portion of this layer becomes separated from the upper portion by a process of delamination, and is turned ventralwards by the continued growth of the optic lobes. The upper portion of this layer maintains its connection with the retinal cells in the optic plate, and with the dorsal wall of the anterior optic invagination; the cells of this portion of the middle layer of the fold elongating as the retina is carried farther away from the anterior invagination by the growth of the fold, forming the fibers of the optic nerve.

I cannot claim that I have established the fact that the nerves of the anterior median eyes arise in this fashion; but careful and repeated examination of my material leads me to believe that these



TEXT-FIGURE 3. Development of the anterior median eye. (Diagrammatic.)

A. Condition of the eye at an early stage in the development of the embryo.

a. o. v., anterior optic vesicle. The lumen of the vesicle, *lu.*, is still conspicuous and opens to the exterior as the semi-circular (crescentic) groove. *o. pl.*, the optic plate. *e. f.*, edge of the cephalic fold. The ectoderm forming the "hood" connects the optic plate with the anterior lip of the optic invagination. *c. l.*, cerebral lobes.

B. At a more advanced stage.

ret., the retina, formed by the involuted optic plate. *cor.*, corneal layer, formed by the ectoderm lying above the retinal portion of the eye. *o. n.*, the optic nerve (rudiment) formed by the line of ectodermal cells which connect the retina with the lip of the anterior optic vesicle. *v. e.*, vesicle of the median eye. The term vesicle is, at this stage, purely nominal, since the lumen of the sac formed by the involution of the optic elements is contiguous with the lumen of the anterior vesicle.

C. The eye at a considerably advanced stage.

The post-retinal layer, *pr. l.*, is formed by certain of the cells which belong to the layer uniting the retina with the anterior optic vesicle becoming connected with the cerebral lobe. By the increased growth of the lobe these cells are turned outward until they come to lie directly beneath the retina, thus forming a third optic layer. The sac formed by the involution of the optic elements is now separated from the lumen of the anterior optic vesicle, which becomes practically obliterated.

That portion of the ectodermal layer which remains attached to the anterior optic vesicle, connecting it with the retinal portion of the eye, *o. n.* in C and D, becomes the optic nerve.

D. A still later stage.

The three layers of the anterior median eye become more closely approximated as its development continues.

The lettering for C and D is the same as in the preceding figures.

nerves can be accounted for in this way. If this should prove to be the case, it will appear that the nerves supplying these eyes are simply modifications of the ectodermal cells which originally serve to connect the retina with the vesicle of the anterior optic sac; and, what is more significant, the anterior median eyes maintain their connection with the optic tracts of the brain throughout the whole process of their formation.

The Posterior Median, and Lateral Eyes.—The accessory eyes appear somewhat later than the principal eyes as simple ectodermal thickenings in the optic area (Fig. 32; *p. m. e.* and *l. e.*). A depression appears in the region of each optic thickening from which these eyes are formed, the walls of which grow inward in such a manner that the thickened portion, or retina, is completely covered. The cells which have grown over the retina in this manner secrete the lens, and have been called, in consequence, the *corneal layer.*

The manner in which the nerves supplying the accessory eyes are formed is not clear. The possibility that the connection of these eyes with the optic lobes is obtained secondarily has to be admitted, although there appears to be little doubt in the minds of those who have investigated the subject that the connection of the lateral eyes with the lateral optic tracts bears some relation to the formation of the lateral optic vesicle.

VII. Comparison of the Araneid Brain with that of Other Arthropods.

Any effort to homologize the brain of *Epeira* with that of other Arachnids, or with the brains of Arthropods in general, is attended with a great deal of difficulty on account of the divergent statements of the different investigators who have studied the subject.

That there is a similarity between the brain of the scorpion and that of the spider is evident from the descriptions both of St. Remy and of Patten. This similarity is to be found not only in the form of the adult brain, but also in its method of development. Patten found three pre-cheliceral segments in the cephalic plate of the spider, each segment consisting of a cerebral ganglion, an optic ganglion, and an optic plate. He also found the same condition to hold true of the cephalic plate of the scorpion.

The optic tracts in the spiders and in the scorpions arise in connection with an anterior optic, and two lateral optic, invaginations. In both forms these invaginations give rise to homologous parts of the optic lobes. In both the spiders and the scorpions the character of the two sets of eyes is similar; the median eyes being formed by a process of infolding which results in the inversion of the retinal elements, in both forms the median eyes being formed, first as simple ectodermal thickenings on the outer borders of the cephalic plate, which, later, become folded over the head to form a cephalic fold that bears the anterior median eyes on its posterior margin.

Other investigators, however, do not all coincide with Patten in his statement concerning the presence of three pre-cheliceral segments in the scorpion. Brauer and Lankester, whose papers appeared before Patten's, report, the former, two such segments, and the latter, one. McClendon, working under the direction of Wheeler, found two segments in the species upon which Patten worked. Police seems to have established the presence of two pre-cheliceral segments in *Euscorpius italicus*.

This divergence of opinion makes the question of comparison one of great difficulty. In *Epeira* the three pre-cheliceral segments are clearly distinguished, particularly in the earlier stages. From the closeness of the relationship of the two forms, one would expect to find the same number in the scorpion. Patten's figures of the cephalic plate of *Buthus* would seem to make it evident that this number exists in that form.

An attempt to homologize the brain of the spider with that of insects is attended with even greater difficulty. Patten records the presence of three pre-oral segments in *Acilius;* the first of these segments is less distinct in this form than in either the spider or the scorpion. Wheeler, in his paper on *Doryphora* (41), believed that he had found three pre-oral segments in that form; but he was led to change his conclusion in a later paper on the morphology of the insect brain (42), in which he states that only two such segments exist.

Viallanes, on the other hand, finds evidence that three pre-oral segments are present in the brain of *Mantis;* and Holingren, in his

paper on the morphology of the insect head, states that, in his opinion, most of the recent investigators incline to recognize that the pre-oral portion of the cephalic plate of insects is composed of three parts, or segments.

Assuming this to be the case, it appears that there are certain lines of comparison by which the Arachnid brain can be homologized with the brains of insects.

1. The rudiment of the brain is laid down on the yolk as a broad cephalic plate in both Arachnids and Insects.

2. This plate, in both groups, presents three transverse segments, anterior to the stomodæal depression, which form the foundation of both the cerebral and optic ganglia.

3. Each of the pre-oral segments, in Insects and Arachnids, presents a cerebral and an optic ganglion, and a marginal limb which bears the retinal elements—the optic plate.

4. The simple eyes of insects arise, as do the lateral eyes of spiders and scorpions, in association with invaginations which appear on the lateral and anterior margins of the cephalic plate. These invaginations form important parts of the optic lobes in both groups.

VIII. General Considerations.

It would appear from the foregoing discussion that the brain of the Arachnida presents a decided advance in complexity of structure, and in the method of its development, over that of the Insecta; or, indeed, any other group of the Arthropoda.

The method by which the cerebral vesicle is formed, and the relation of the anterior median eyes to this structure, bears a striking resemblance to the formation of the cerebral vesicle, and the growth of the pineal eye, in vertebrates, as has been pointed out by Patten in his paper on the Origin of the Vertebrates.

It has been objected that the presence of two median eyes, which are separate structures in the Arachnid head, would necessitate a double origin for the pineal organ in vertebrates, no trace of which can be said to exist. This objection has lost some of its force since the publication, by Locy, of a paper in which he shows that, in the Elasmobranchs, the pineal eye is formed by the union of two inde-

pendent optic rudiments which arise on the margin of the cephalic fold.

This question will not be settled by the study of the Araneid brain alone but by a very careful comparison of typical Arachnids on the one hand, and of the Vertebrata on the other. The suggestions concerning the origin of the Vertebrata, which arise from these considerations, demand that the problem should receive the most careful attention.

Acknowledgments.

I take this opportunity for acknowledging my indebtedness to my friend and teacher, Dr. William Patten, at whose suggestion this study was undertaken, and under whose guidance it has been accomplished. I also take pleasure in recognizing the kindness of the trustees of the Carnegie Institute, who placed a room at the Biological Laboratory at Wood's Hole at my disposal for the summer of 1905, at which place a considerable portion of the work presented in this paper was done.

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LETTERING OF PLATES

A. E., anterior end.
a. m. e., anterior median eyes.
a. f., amniotic fold.
ant. gr., anterior groove.
ant. ves., anterior vesicle.
ap., appendages.
bld., blastodisc.
blp., blastopore.
br., brain.
chl., chelicera.
chl. c., coxal portion of chelicera.
chl. gl., cheliceral gland.
chl. seg., cheliceral segment.
cht., chitinous covering.
c. gl., cerebral ganglia.
c. pl., cephalic plate.
c. th., caudal thickening.
ec. f., ectodermal furrow.
ect., ectoderm.
e. p., ectodermal pit.
f. c., fat cells.
g. b., germ band.
gl., gland.
ht., heart.
lat. gr., lateral groove.
l. e., lateral eye.
l. fr., lateral furrow.
ls., lens.
l. ves., lateral vesicle.
md. pdp., mandibular part of pedipalp.
m. e., median eye.
m. f., median furrow.
m. o. p., middle optic pit.
msd., mesoderm.
mth., mouth.
mus., muscle.
n. b., neuroblast.
n. c., cord.
n. sp., nuclear spindle.
nu., nucleus.
oes., œsophagus.
o. f., optic fold.
o. gl., optic ganglion.
o. pl., optic plate.
o. n., optic nerve.

pdp., pedipalps.
pdp. c., coxal portion of the pedipalps.
pdp. gl., pedipalpal gland.
pdp. seg., segment of pedipalps.
P. E., posterior end.
p. m. e., posterior median eyes.
p. s., *punktsubstanz.*
ros., rostrum.
ros. n., rostral nerve.
s. c., supra-stomodæal connective.
seg., segments.
s. s. c., sub-stomodæal connective.
st., stomodæum.
y. c., yolk cells.
ylk., yolk.

DESCRIPTION OF PLATES.

PLATE I.

FIG. 1. Early stage in the formation of the blastoderm. The nuclei, *nu.*, formed from the division of the segmentation nucleus, having migrated to the surface of the egg, lie in the periplasm just outside the yolk columns, *ylk.*

FIG. 2. Blastodisc. The division of the egg into two poles is indicated by the aggregation of cells, as indicated by their nuclei, on one end of the egg. Irregularly projecting yolk masses and fewer nuclei, mark the other end. A slight, blastopore-like depression, *blp.*, appears in the center of the blastodisc.

FIG. 3. Blastodisc, viewed from above.

FIG. 4. Primary thickening, viewed from above. The increased thickness of the germ layer is indicated by the closely aggregated character of the nuclei.

FIG. 5. Primary thickening, viewed from the side. The slight elevation to one side of the blastopore is the caudal thickening, *c. th.*

FIG. 6. Germ band. The germ band, *g. bd.*, lies in the form of a triangular plate on the surface of the egg. It bears, at intervals, thickened, transverse ridges, or segments, *seg.* The most posterior portion of the plate is occupied by the caudal thickening. The anterior portion consists of the flat cephalic plate, *c. pl.*

FIG. 7. A more posterior view of the same.

FIG. 8. The germ band. A somewhat earlier stage, presenting fewer segments.

FIG. 9. The ventral plate. A much later period, presenting a much greater number of segments, the thoracic segments bearing rudimentary appendages, *ap.*

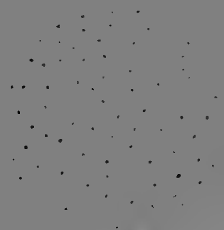


FIG. 1.

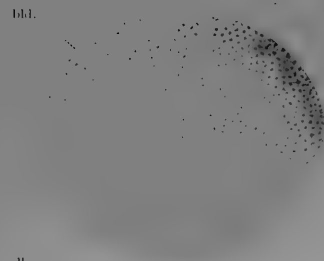


FIG. 2.

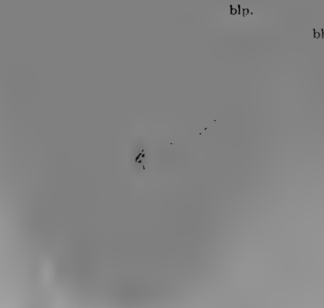


FIG. 3.

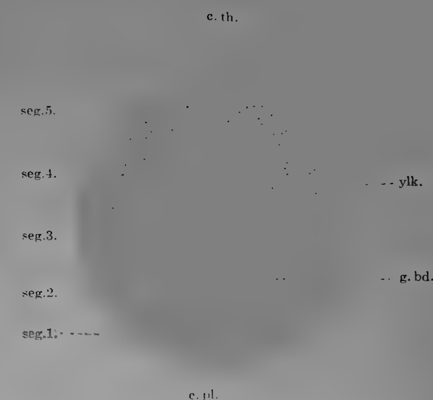


FIG. 6.

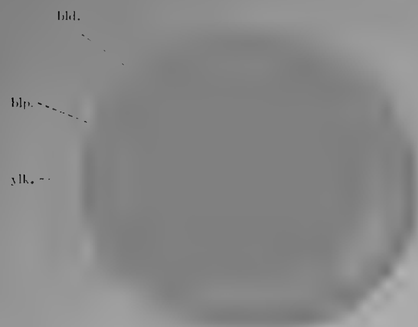


FIG. 4.

FIG. 5.

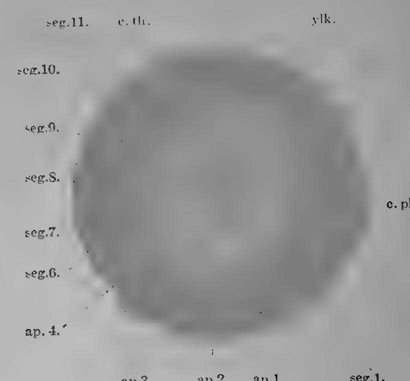


FIG. 9.

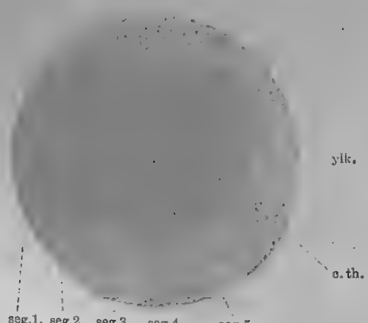


FIG. 7.

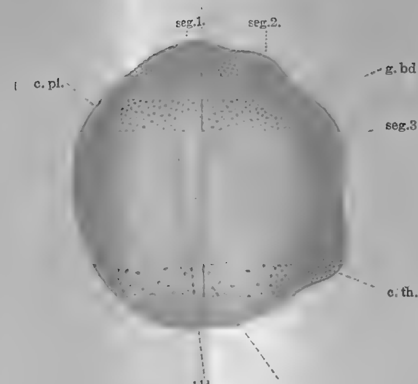


FIG. 8.

PLATE II.

FIG. 10. Primitive cumulus stage. Transverse section through the egg, showing the manner in which the mesoderm is formed, *msd.*, beneath the blastopore, *blp.* The beginning of the endoderm is seen where a few cells are being forced into the yolk, *y. c.*

FIG. 11. Blastodisc. Longitudinal section through egg, showing the origin of the mesoderm by proliferation of cells from the ectoderm, *ect.*, in posterior, or caudal, region.

FIG. 12. A similar section a little to one side of the median line.

FIG. 13. Cells from the blastodisc, showing the vesicular character of their protoplasm.

FIG. 14. Cells from the blastodisc, showing a nuclear spindle, *n. sp.*, which indicates the plane of division of the cells in this region.

FIG. 10.

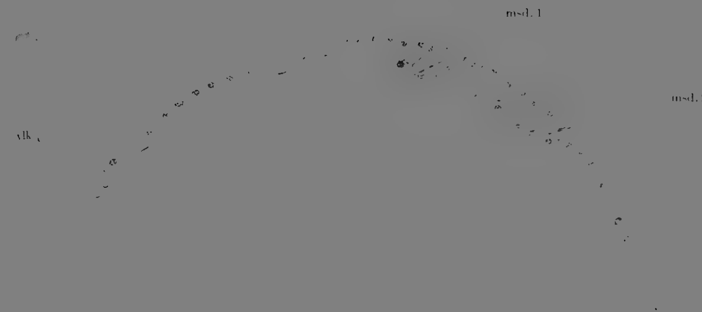


FIG. 11.

FIG. 12.

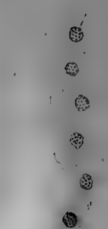


FIG. 13.

FIG. 14.

PLATE III.

FIGS. 15 and 16. Longitudinal sections through the blastodisc a little to one side of the median line, showing, still further, the origin of the mesoderm cells from the caudal region, and the migration of endoderm cells into the yolk.

FIG. 15.

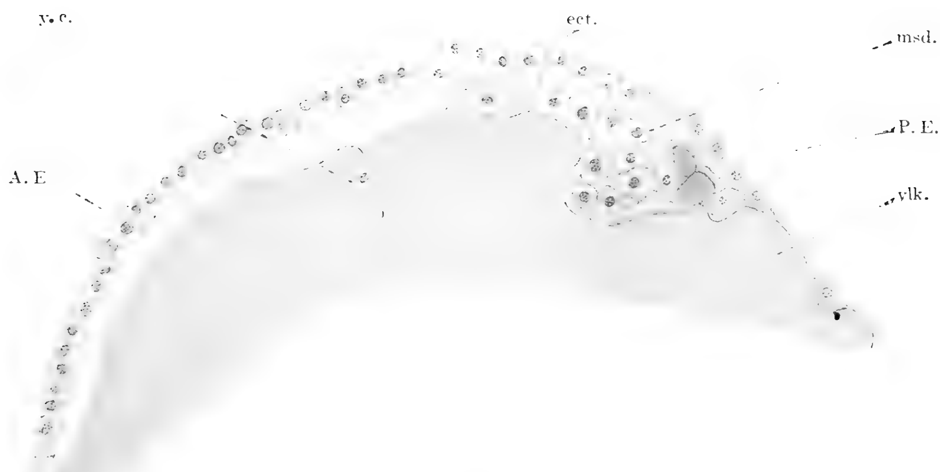


FIG. 16.

PLATE IV.

FIGS. 17 and 18. Longitudinal sections through the embryo at the beginning of the germ band stage, showing the position of the caudal thickening, *c. th.*, immediately behind the blastopore, *bpl.*, the mesoderm, *msd.*, lying just beneath the outer cell layer, *ect.*, and the yolk cells, *y. c.*, which contribute to the formation of the endoderm.

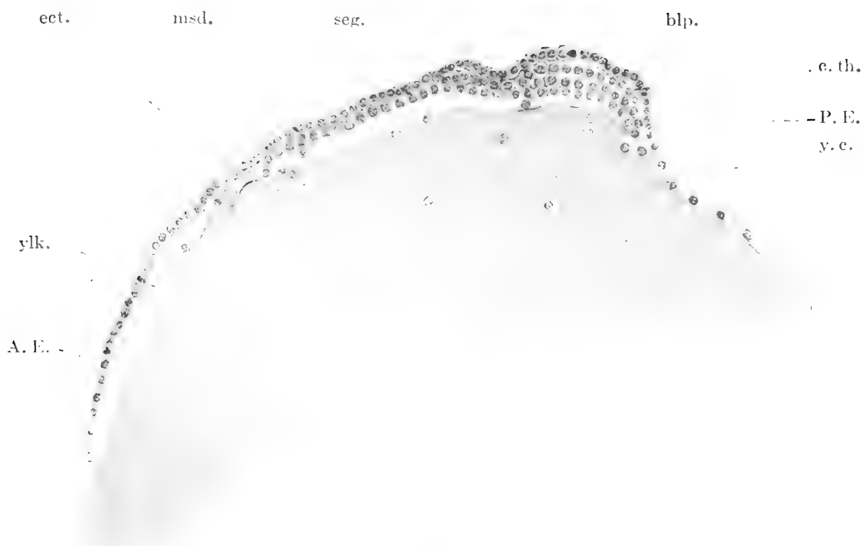

ect.
msd.
seg.
blp.
c. th.
P. E.
y. c.
ylk.
A. E.

FIG. 17.

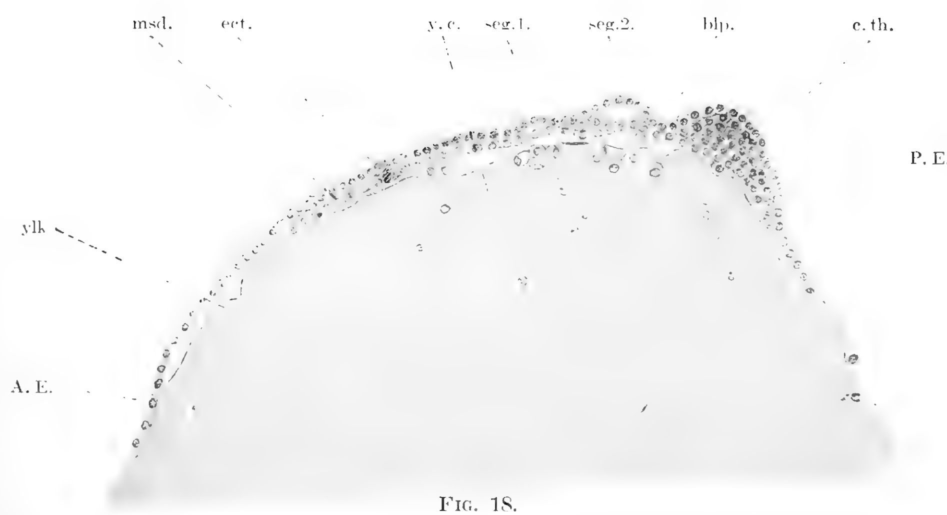

msd.
ect.
y. c.
seg. 1.
seg. 2.
blp.
c. th.
P. E.
ylk
A. E.

FIG. 18.

Plate V.

Fig. 19. Cephalic plate of *Epeira*. Stage I. Showing the thickened areas which form the beginning of the optic ganglia, *o. gl.*, and the rostrum, *ros.*. the rudiments of the cheliceræ, *chl.* and pedipalps, *pdp.*, and the lateral groove, *lat. gr.*

Fig. 20, Stage II. Showing a slight advance in the differentiation of the optic ganglia and in the development of the rostrum; also a marked increase in the growth of the pedipalps and cheliceræ.

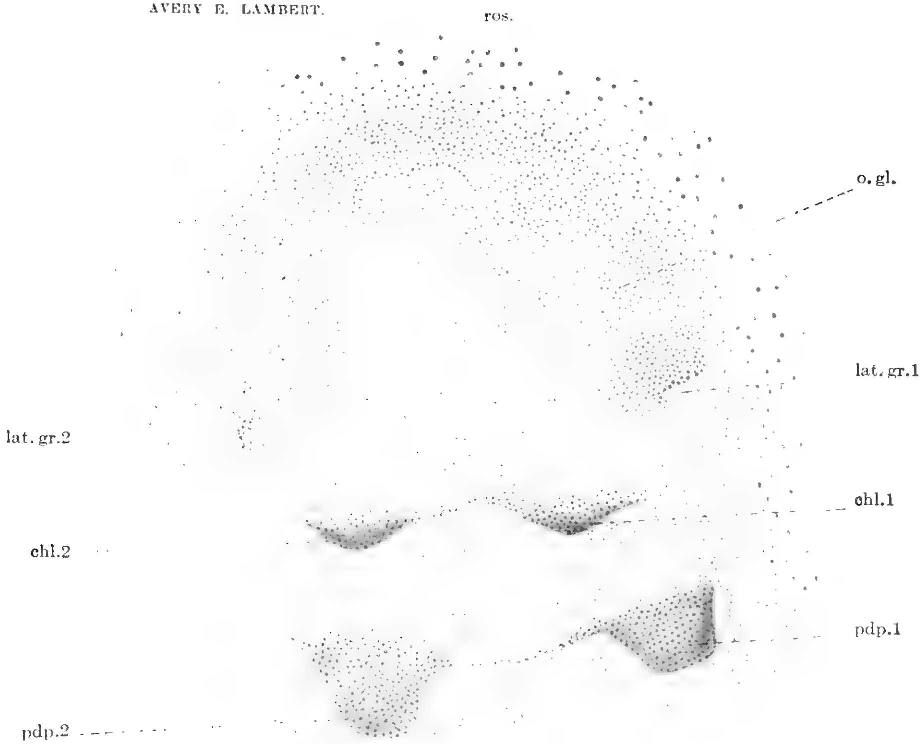

ros.
o. gl.
lat. gr.1
lat. gr.2
chl.1
chl.2
pdp.1
pdp.2

FIG. 19.

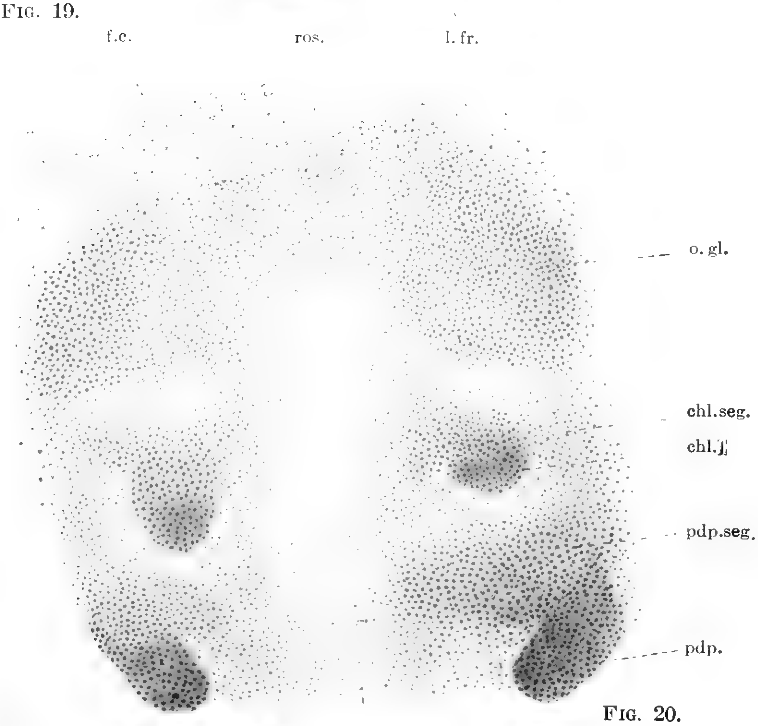

f.c.
ros.
l. fr.
o. gl.
chl.seg.
chl.
pdp.seg.
pdp.
l.fr.

FIG. 20.

Plate VI.

Fig. 21, Stage III. Showing increased differentiation in the optic area, and the double character of the rudiment from which the rostrum is derived.

Fig. 22. Showing some advance over Fig. 21. The cerebral ganglia, *c. gl.*, and optic ganglia, *o. gl.*, are more distinctly differentiated, the rostrum has become more prominent, as is also the stomodæal depression.

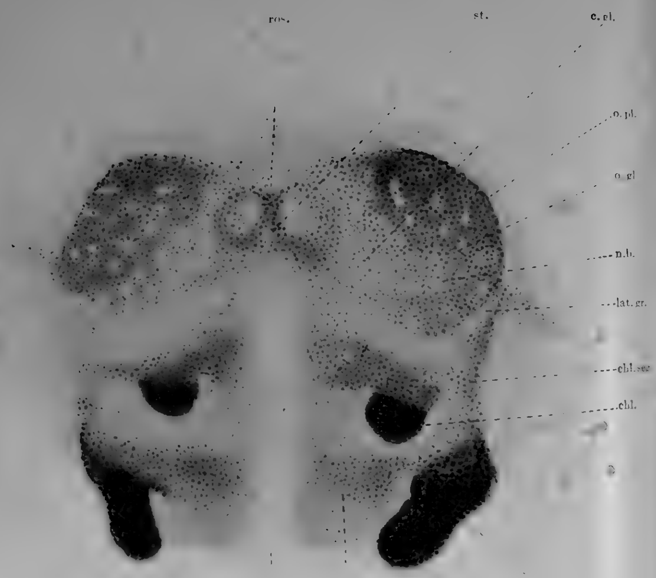

ros.
st.
c. gl.
o. pl.
o. gl
n.b.
lat. gr.
chl. seg.
chl.
l. fr.
pdp. seg.
pdp.

FIG. 21.


c. gl.1.
f. c.2.
ros.
ant. gr.
f. c.1.
st.
n. b
c. gl 2.
lat gr.
chl.
pdp.seg.
l. fr.
pdp.

FIG. 22.

Plate VII.

Fig. 23, Stage IV. Showing the partial coalescence of the two parts of the rostral rudiment, the extension of the optic plate, *o. pl.*, the formation of a distinct coxal portion to the pedipalps, *pdp. c.*, which forms the rudiment of the mandibles. The increase in the number of neuroblasts in the cerebral and optic areas, and their extension to the neuromeres of the cheliceræ, pedipalps, and the thoracic appendages, is apparent.

Fig. 24, Stage V. The advances made in the development of the cephalic plate are shown in the deepening of the anterior groove, *ant. gr.*, and of the lateral grooves, *lat. gr.*, the shifting of the rostrum from the anterior margin of the plate to a point between the cerebral ganglia, the more complete union of the parts of the rostrum, and the beginning of the fold which forms the roof of the cerebral vesicle, *o. f.*


ros. n.
ros.
f. c.
st.
c. gl.1
o. gl.1
o. pl.
c. gl 2.
o. gl.2
c. gl.3
o. gl.3
lat. gr.
s. s. c.
n. b.1
n. b.2
chl. seg.
chl. c.
chl.
pdp. c.
pdp.seg.
l. fr.
pdp.
FIG. 23.


o.gl.2
ant.gr.1
c.gl.1
ros.
s.c.
st.
ant.gr.1
c. gl.2
o. pl.
o. f.
c. gl.2.
s. s. c.
o.gl.2
n. b.
c.gl.3
lat. gr.
chl. seg.
o. gl.3
chl.
md. pdp.
n. c.
pdp.
FIG. 24.

Plate VIII.

Figs. 25 and 26. Showing the complete coalescence of the parts of the rostrum to form the flat rostral plate, *ros.*, the formation of a mandibular segment on the base of the pedipalp, *md. pdp.*, the partial separation of the neuroblasts in the cerebral and optic lobes as indicated by their fractured appearance, the deepening of the anterior and lateral grooves, and a considerable increase in the growth of the optic fold, *o. f.*

The condition shown in Fig. 25 is somewhat abnormal and is not so far advanced as that shown in Fig. 26.

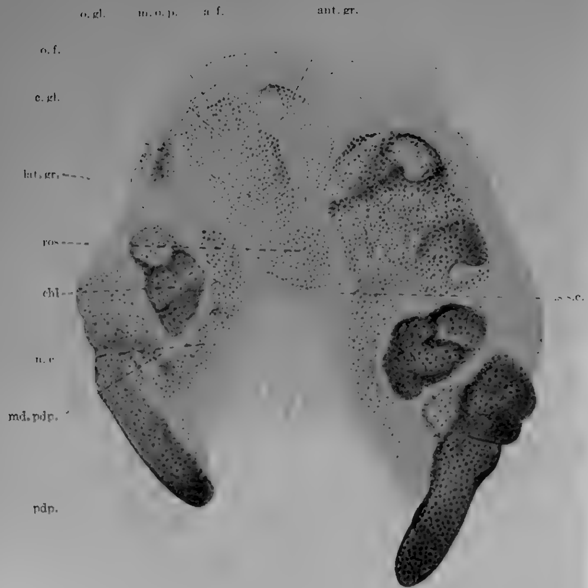

o. gl.
m. o. p.
a. f.
ant. gr.
o. f.
c. gl.
lat. gr.
ros
chl
s. s. c.
n. c
md. pdp.
pdp.

Fig. 25

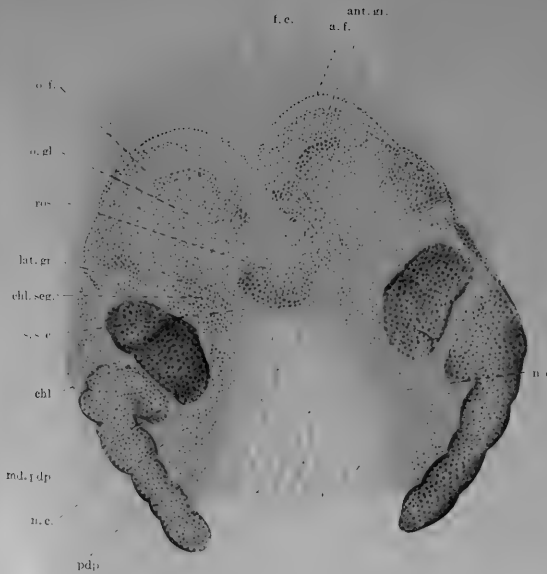

f. c.
ant. gr.
a. f.
o. f.
o. gl
ros
lat. gr
chl. seg.
s. s. c
chl
n. c
md. pdp
n. c.
pdp

Fig. 26.

Plate IX.

Fig. 27. Stage VII. Showing the union of the cerebral and optic ganglia to form the pro-cephalic lobes, the deepening of the anterior and lateral optic invaginations to form vesicles, the optic thickening which forms the rudiment of the lateral eyes, *l. e.*, the increased growth of the optic fold, the shifting of the rostrum posteriorly until it completely covers the stomodæum which is flanked on either side by the neuromeres of the cheliceral segment, also the ectodermal pit, *e. p.*

Fig. 28. Stage VIII. Showing the narrowing of the anterior portion of the head region, the increased growth of the optic fold, and the general concrescence of the optic and cerebral ganglia.

AVERY E. LAMBERT.

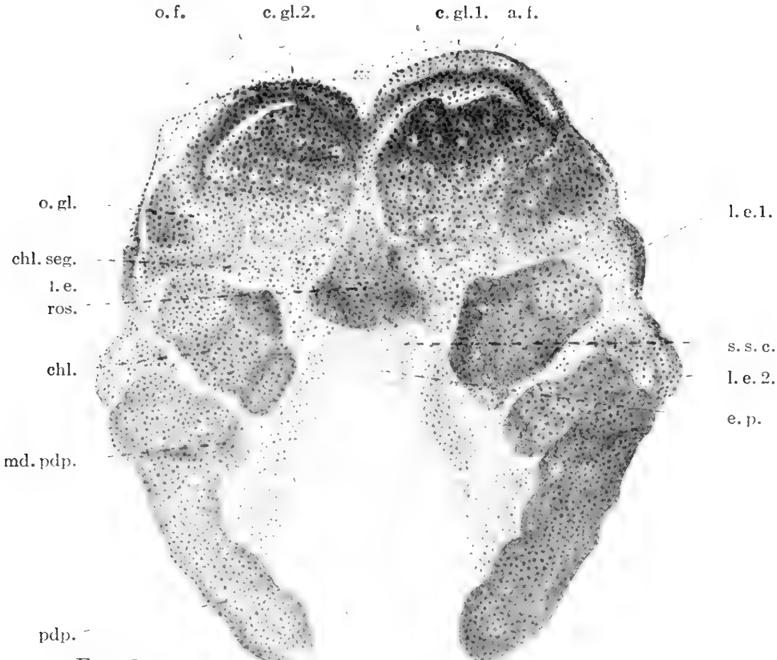


FIG. 27.

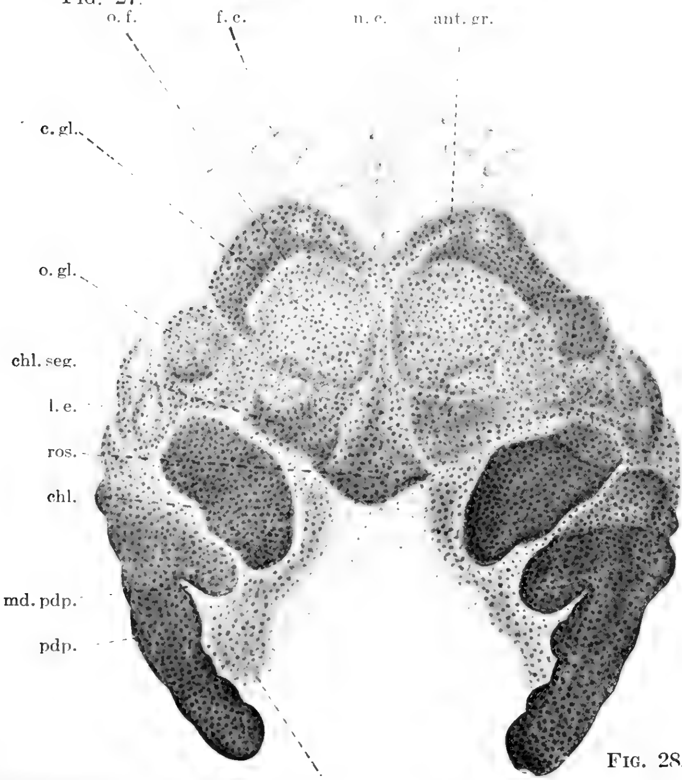


FIG. 28.

PLATE X.

FIG. 29. Showing the conditions a little more advanced than in the preceding figure, the optic fold covering a considerably greater portion of the cephalic plate. The presence of the ectodermal pit is indicated, *c. p.*, which tends to extend as a furrow in the direction of the stomodæum.

FIG. 30. Stage IX. Showing the increased growth and fusion of the anterior portions of the optic fold which now nearly covers the optic and cerebral ganglia, the position of the cheliceræ which have come to lie on either side of the rostrum, the closure of the anterior and lateral grooves completing the formation of the anterior and lateral vesicles, and the extension of the ectodermal pit to form the ectodermal furrow, *ec. f.*

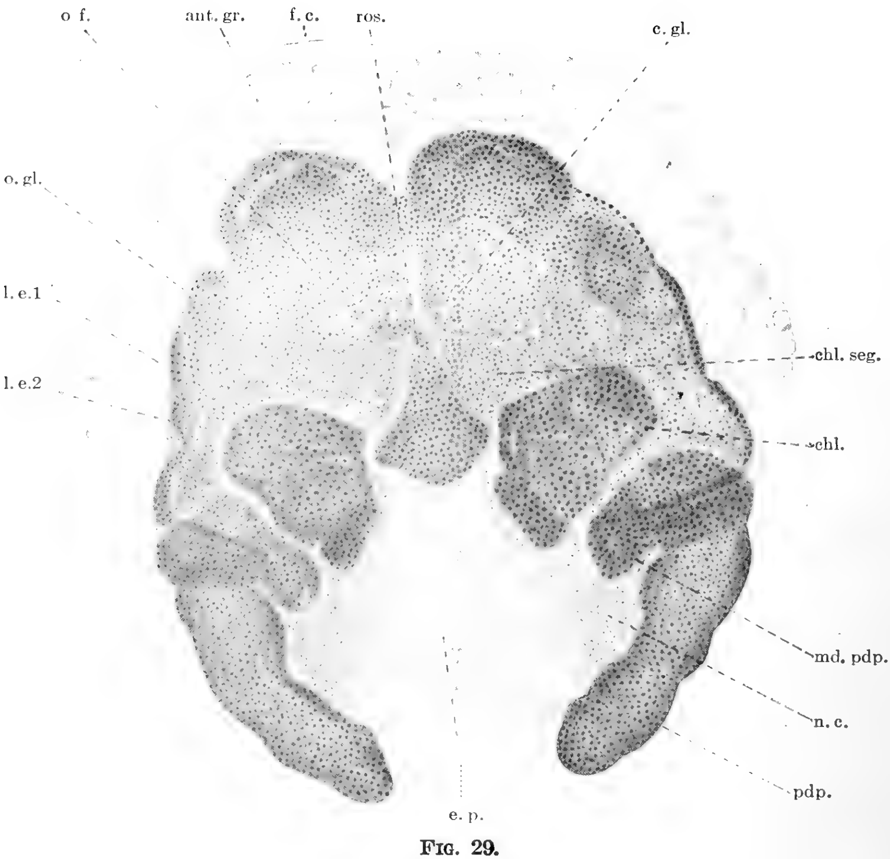


FIG. 29.

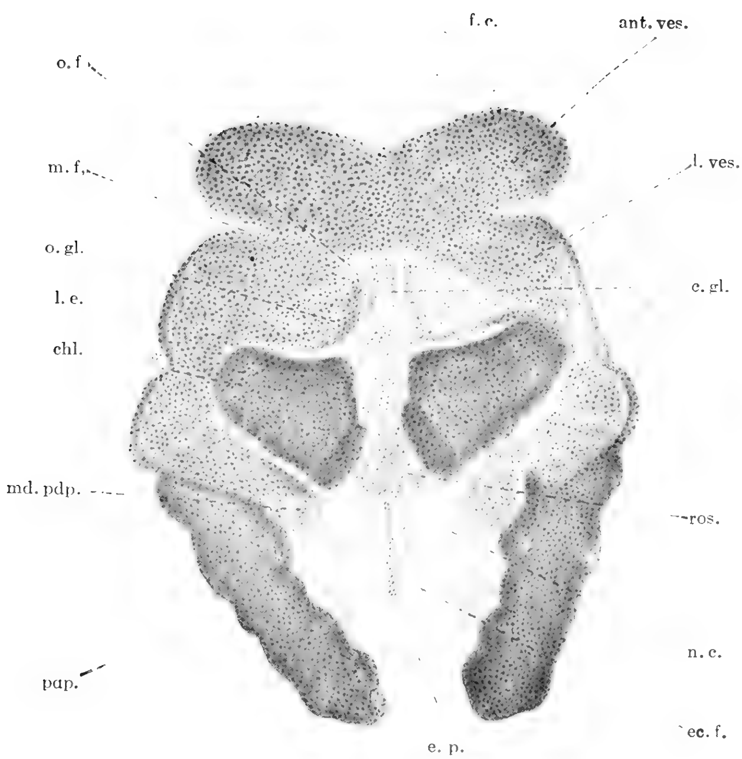


FIG. 30.

PLATE XI

FIG. 31. Stage X. Showing the optic fold now covering the entire area of the optic and cerebral ganglia, which also bears the anterior median eyes, *a. m. e.*, on its edge, also the closed ectodermal furrow, *ec. f.*

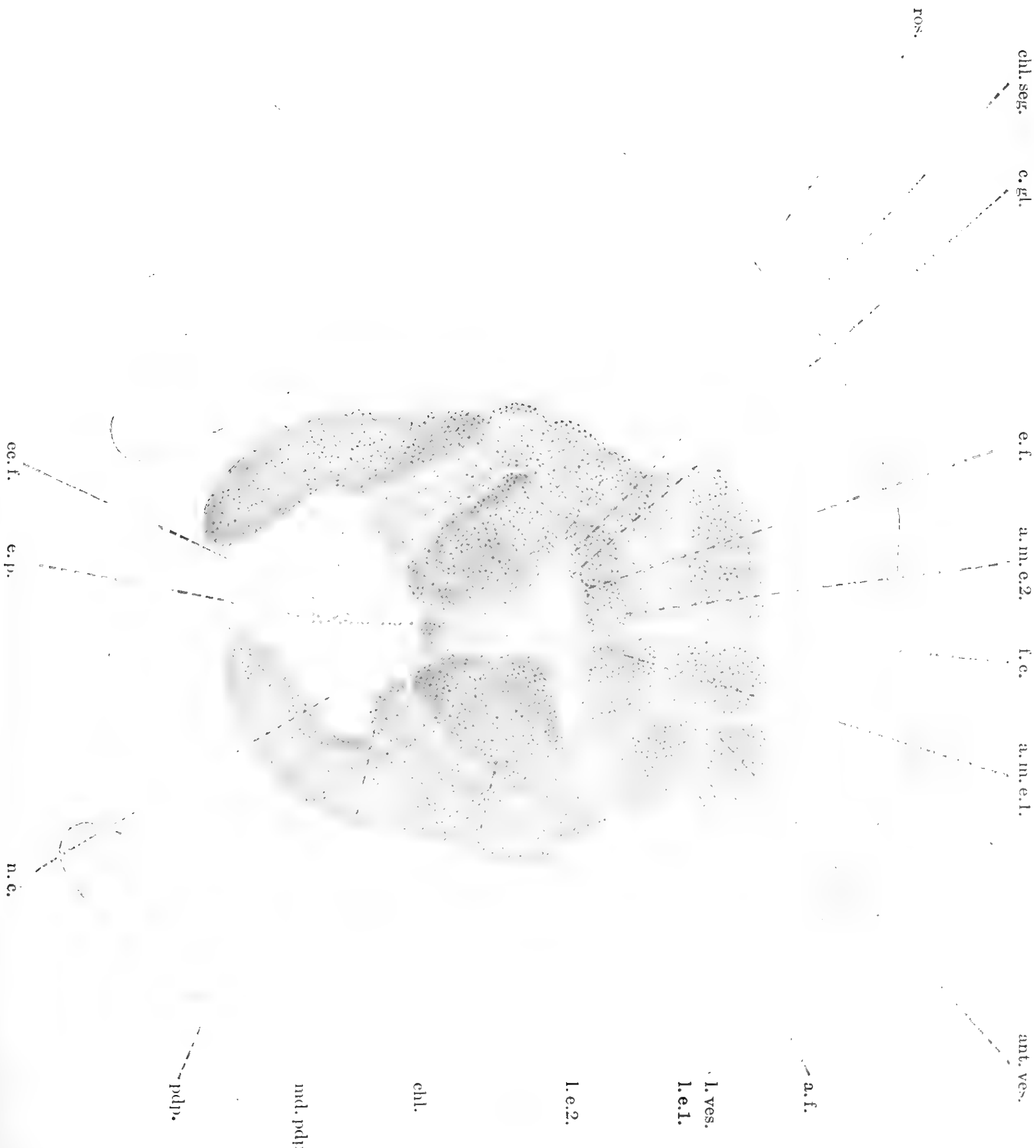

ros.
chl. seg.
c. gl.
e. f.
a. m. e. 2.
f. c.
a. m. e. 1.
ant. ves.
a. f.
l. ves.
l. e. 1.
l. e. 2.
chl.
md. pdp.
pdp.
n. c.
c. p.
cc. f.

Fig. 31.

Plate XII.

Fig. 32. Stage XI. Showing the condition of the cerebral lobes in an embryo about to hatch.


pdp.gl.
ros.
pdp.
md. pdp.
chl.
chl. gl.
l. e.
a. m. e.
p. m. e.
l. ves.
ant. ves.
c. gl.
ect.
ht.

FIG. 32.

Plate XIII.

Figs. 33 and 34. Longitudinal sections through the head of a young spider about to hatch.

Fig. 33. Showing the median eyes, and the brain which occupies a relatively greater portion of the cephalothorax than is the case with the adult spider.

Fig. 34. Showing the mouth, *mth.;* œsophagus, *œs.;* the cheliceral muscles, *mus.;* the procephalon, *br.;* and the postcephalon, *s. o. g.*, 1-12.


m.e.2
br.3
br.2
cht.
br.1
ps.
op.n.
m.e.1
ls.
ret.
pdp.gl.
chl.mus.
ros.
mth.
s.o.g.
cht.
ect.
mus.

FIG. 33.


mus.
m.e.
oe.
gl.
mth.
ps.
br.
cht.
mus.
s.o.g.1
s.o.g.2
s.o.g.3
s.o.g.4
s.o.g.5
s.o.g.6
s.o.g.7
s.o.g.8
cht.
s.o.g.9
s.o.g.10
ect.
s.o.g.11
s.o.g.12

FIG. 34.

COMPARATIVE STUDIES IN CRUSTACEAN SPERMATOGENESIS.

M. LOUISE NICHOLS.

WITH 3 PLATES AND 12 TEXT FIGURES.

This study was undertaken in order to determine how far types of the class Crustacea, showing in the adult condition well marked morphological differences, would also present in the development of the germ-cells characteristic dissimilarity. Accordingly, an examination was made of the following forms: the Amphipod, Talorchestia longicornis, the Isopods, Oniscus asellus Linn. and Idotea irrorata, the Decapods, Hippa talpoides, Astacus fluviatilis and Homarus vulgaris.

There is much variety among the Crustacea with regard to the appearance of the spermatozoa and an increasing complexity of structure from the lower to the higher forms. A comparison of the spermatozoa makes, therefore, a convenient beginning for a study of this nature.

SPERMATOZOA.

Entomostraca. Phyllopoda. Zacharias (1893) has studied the spermatozoa of the Daphnid Polyphemus. They are amœboid in shape, the character of the pseudopodia varying with the nature and density of the solution in which they are placed. In a cell so simple, of course no distinction can be made between head, middle-piece and tail. (Text Fig. 1.)

Cirripedia. In the spermatozoa of Lepas anatifera L. and Balanus improvisus Darw. Ballowitz (1908) was unable to discover any part taking the characteristic nuclear stain of alum carmine. The cell by teasing, however, could be separated into fibrils one of

which stained intensely with gentian violet. It seems probable that this represents the head, the pale fibres the tail. (Text Fig. 2.)

Copepoda. McClendon (1907) describes the spermatozoon as very similar to those of the Cirripedia.

Malacostraca. Arthrostraca (Edriophthalmata). Those which have been studied possess the flagellate type of spermatozoon. The head and tail are sharply marked off, although sometimes no middle-piece can be distinguished. Relations of size and position between head and tail differ somewhat in the members of the group. A simple rounded head followed by a tail of moderate length, and in Talorchestia by a middle-piece, characterize the Amphipoda, while in the Isopoda both head and tail are elongated, the latter excessively so. The head and tail also, instead of joining in a straight line as is the case with the Amphipoda, form with each other an acute angle, as Gilson (1886) has described it, much like the handle of a whip and its lash. The general appearance of the spermatozoa resembles that of the Cirripedia. (Text Figs. 3, 4.)

Thoracostraca (Podophthalmata). The spermatozoon of Mysis as described by Gilson (1886) is very like that of the Cirripedia and the Isopoda, the head and tail occupying similar relative positions, but that of Squilla is quite different, seems indeed to forecast the spermatozoon of the Decapoda. It is spherical in shape, the greater part of the cell consisting of a colorless vesicle filled with hyaline substance, while the only portion staining with methyl green is a button- or lens-shaped body at one pole of the sphere. (Text Figs. 5, 6.)

Grobben (1906), Koltzoff and others have shown for the Decapoda that the more nearly related adult members are, the more nearly alike is the structure of the spermatozoa. Koltzoff (1906) especially has worked out this subject with some care and has outlined a genealogy of the Decapoda based on this feature.

The characteristic most prominent in the Decapod spermatozoon is the development of a resistant capsule in the region of the tail (S. vesiculifera of Koltzoff). In the lower forms this is represented by a spine-like projection (S. anacantha). (Text Fig. 7.) In the higher forms it is more vesicular in shape and in the region

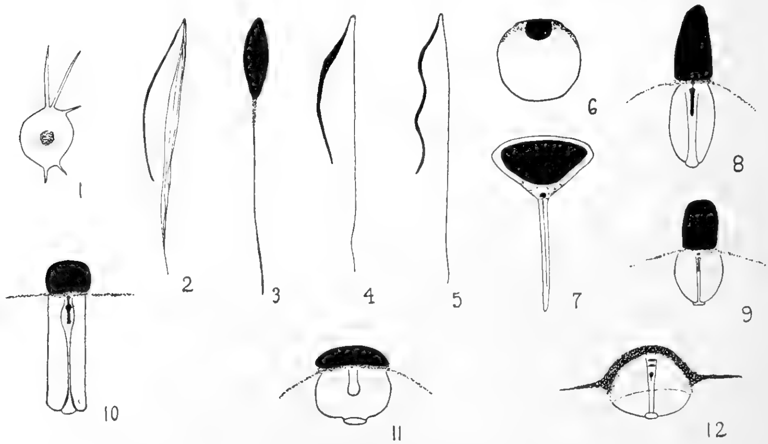
of head or middle-piece are present projections or streamers which are entirely missing in the S. anacantha (Text Figs. 8-12). If these projections have their origin in the region of the neck the spermatozoon belongs to the S. ancanthina deracantha, represented by the Galatheidæ and Paguridæ, whereas if they come from the head region it is classified as S. cephalacantha, represented by the Brachyura, Dromiidæ and Oxystomata (Text Figs. 8-12). In the latter forms the tail capsule is more or less drawn into and surrounded by the head region which gives the spermatozoon a contracted appearance and causes it to approach more nearly a spherical shape. According to Koltzoff's classification, the spermatozoa of Astacus, the Loricata and the Thallassemidea are placed in a separate group, the S. contracta, characterized by a shortening and broadening of the tail capsule. No very sharp line can, however, be drawn between these forms and the Galatheidæ and the Paguridæ, the dimensions of the tail capsule varying within comparatively wide limits.

In those Decapods which have been carefully studied the tail capsule contains a narrow tube or vesicle within which is to be found the centrosome and its derivatives. The proximal centrosome lies anteriorly in the region of the processes, while a distal, elongated and otherwise modified centrosome lies behind it extending into the tube. (Text Figs. 8, 9, 10, 12.) A comparison with the flagellate type leads to the conclusion that the nucleus may be regarded as the head, the region of the processes as the middle-piece, the capsule as the tail, while the modified distal centrosome may correspond to the axial filament. The space between the inner tube and the outer wall of the capsule is believed by Koltzoff to be occupied by an explosive substance.

The homologies of the Decapod spermatozoon have only recently been satisfactorily elucidated. Auerbach (1895) interpreted the parts of the spermatozoon much as has been outlined above, but did not work out the details. As late as 1904 Labbé described the capsule as anterior in position to the nucleus and processes and investing the acrosome. He believed it to be present only in the immature spermatozoon and that as a final stage of development the nucleus

became invaginated or surrounded in an amniotic fashion by the capsule. The latter then disintegrated, leaving as the mature spermatozoon only the nucleus, processes and "acrosome." Koltzoff, however, by his careful and detailed study of the structure and behavior of the spermatozoa has shown very conclusively that this method of homologizing the parts of the spermatozoon is consonant neither with comparative morphology nor with the behavior of the spermatozoon in fertilization.

One of the most unique and puzzling features of the Decapod spermatozoon is its lack of motility. The observations which Koltzoff was able to make on the method of fertilization throw some light



TEXT-FIGURES 1-12.

Comparative morphology of Crustacean Spermatozoa (somewhat diagrammatic).

1, Polyphemus; 2, Cirripedia; 3, Amphipoda; 4, Isopoda; 5, Mysis; 6, Squilla; 7, Carididae; 8, Anomura; 9, Anomura; 10, Homarus; 11, Astacus; 12, Brachyura.

on this subject. An examination of a mixture of the eggs and spermatozoa of Galathea revealed the fact that the spermatozoa are carried passively by a stream of water to the surface of the egg. When a protoplasmic process touches the surface of the egg it becomes firmly attached. The rest of the spermatozoon continues to sway back and forth until the other processes are brought in contact

with the egg, thus orienting the spermatozoon. The processes then contract until the nucleus touches the circumference of the egg. The attachment of the processes is possibly by the excretion of some adhesive substance, but also mechanical; the egg membrane is finely porous, and into the pores portions of the processes insert themselves. Koltzoff believes that the explosion of the capsule then forces the nucleus and the proximal centrosome into the egg, the derivatives of the distal centrosome and the processes being cast off. These observations lend additional weight to the homology outlined above; the capsule fulfills the function of a tail, namely, that of conveying the nucleus and centrosome into the egg.

Development of the Spermatzoon from the Spermatid.

Koltzoff has described with much interesting detail the development of the Decapod spermatid. He traces the origin of the middle-piece and tail from the mitochondrial granules and follows the peculiar development of the distal centrosome within the inner tube of the capsule.

Hippa is the only Decapod from which I obtained a complete series of stages illustrating the transformation of the spermatid. The fixing fluid employed, namely Flemming's strong solution, does not bring out well either the mitochondrial granules or the distal centrosome. According to Benda (1903), the proportion of acetic acid in this fluid is great enough to cause swelling and disappearance of the mitochondrial granules. It is probable, however, that in an animal so nearly related to those described by Koltzoff their development would be much the same. Of the forms described by Koltzoff, that most nearly related to Hippa is Pagurus striatus. Compared with this, the spermatozoon of Hippa is more contracted; both the head and capsule are broader and shorter and the demarcation between head and middle-piece is less sharp. In these respects it approaches more nearly to the S. contracta of the Brachyura. The inner tube of the capsule is closed at its distal extremity by a plug. In my preparations of Hippa the tube is much narrower than in Koltzoff's figure of Pagurus and in the immediate vicinity of the plug the wall of the capsule is somewhat thickened. (Pl. 3, Hi, 34.)

The reduction in size and especially in length of the spermatid in the course of its development is very marked. (Pl. 3. Hi, 29-34.) Its apearance as shown in Figs. 27-29 brings to mind the mature spermatozoon of Homarus, except of course for the greater relative size of the head. It is possible that there is here recapitulated a stage in the phylogeny of the spermatozoon of Hippa.

The spermatid goes through a remarkable series of transformations during its development. The chromatin in the nuclei of the young spermatids is arranged in bands forming a coarse meshwork with large interspaces. (Fig. Hi, 22.) The cytoplasm, at first uniform, soon shows a dense ring around the nucleus. The remainder of the cytoplasm loses the appearance of a network and no longer stains with iron hæmatoxylin. (Fig. Hi, 24.) Koltzoff, in his beautiful Figs. 1-3, Pl. XVI, shows the origin of this differentiation of the cytoplasm in the accumulation and segregation of corpuscles differing from each other in size and in staining capacity, to both of which he gives the name mitochondria. The smaller and darker granules form a mass adjoining the nucleus and correspond to the ring just described for Hippa. They are destined to form the middle-piece of the mature spermatozoon, while the larger and lighter corpuscles coalesce to form the tail capsule. The fixation employed for Hippa does not serve to bring out these granules, but the differentiation of the cytoplasm is clear. In rare instances the mitochondrial body may lie side by side with the nucleus within the cell (Fig. Hi, 23), but in general its appearance is that of Fig. Hi, 24.

Within the mitochondrial body and at one side of the nucleus arise thickened strands which extend to the periphery of the cell. They will become the streamers or projections from the middle-piece of the mature spermatozoon. As the ring increases in diameter and decreases in thickness the strands are gradually more uniformly distributed and no longer appear to arise from so circumscribed an area. (Fig. Hi, 25.) At the same time the nucleus has increased to about twice its former size, the bands of chromatin have nearly disappeared and the entire nucleus stains much more faintly.

The tail capsule has taken a more definite shape, but remains unstained. (Fig. Hi, 25a.) It next commences to elongate, a cavity

is formed in the interior, and at the distal extremity an intensely staining body is conspicuous. (Figs. Hi, 26-27.) The exact manner of formation of the inner tube is a little obscure. Koltzoff (1906) believes that the body (x) takes part in the process. Fig. Hi, 26 might lend itself to such an interpretation. During the formation of the tube only a portion of the body (x) stains brilliantly; the remainder apparently streams towards the centre of the capsule to meet that portion of the tube which is projecting from the proximal end of the capsule. Upon completion of the tube, however, the entire body stains deeply black with hæmatoxylin and becomes constricted into two unequal parts. (Fig. Hi, 27.) But this condition is not retained. The proximal portion (x) separates more completely from the distal and expands into a ring. The distal portion (x) on the other hand swells and comes to stain more diffusely. (Fig. Hi, 28.) In its later development it grows more compact, but its substance seems to retain a considerable degree of plasticity, for it presents much variety of shape. At times it appears to be partially withdrawn into the tube. (Figs. Hi, 30 a-d.) The rest of the capsule is at first quite colorless, but as it develops, longitudinal striations are formed which persist for some time. (Figs. Hi, 29-31.) Fig. 31 shows these striations as two broad, deeply staining bands. In preparations of the stage represented by Fig. 30 which have been considerably destained the striations near the surface of the capsule disappear, but in cross-sections four sharply staining dots indicate striations of the inner tube. (Figs. 30, e-f.)

The nucleus up to this point has remained nearly colorless, but it now becomes much condensed and stains vividly with saffranin, while the middle-piece takes malachite green. The capsule has become short and broad, striations have quite disappeared and the ring and plug, products of the body (x) no longer stain intensely. (Pl. 3, Hi, 32-34.)

A few stages in the development of the spermatid of Homarus were obtained, the material having been preserved and stained in the same manner as the material from Hippa. Figs. Ho, 8, 10 show two stages in the formation of the inner tube. In Fig. 8 there is as yet no wall formed marking off an inner tube within the cap-

sule. Material, however, seems to have collected close to the wall of the capsule. No ring is present at the distal end and the nucleus is condensed, flattened and surrounded by the mitochondrial substance. Enclosed in a vesicle just posterior to the middle-piece lies a densely staining body, probably the distal centrosome. In Fig. 10 the inner tube is complete, a ring has appeared at the distal end and the entire capsule has elongated. Cross sections (Figs. 9 a-b) give some idea of the manner of formation of the tube, apparently by a condensation of the capsular material which still remains connected with the outer wall by delicate strands.

Likewise in the development of the spermatid of Eupagurus longicarpus a few stages were studied. A "Nebenkern" appears in the youngest of them. It increases considerably in size as the nucleus stains more deeply and finally takes its position between the nucleus and the tail-capsule as the middle-piece. A densely staining globule appears at the distal end of the capsule similar to that of Hippa, but much smaller. The smaller size of the spermatid of Eupagurus at this stage is also noticeable and the more densely colorable nature of the nucleus. (Pl. 3. E, 1-5.)

Among the Carididæ I examined only Palæmonetes. The cell walls between adjacent spermatids break down and the nuclei lie in a common cytoplasm. Three such nuclei are represented in Figs. P, 1-3. Pl. 3. In the first the chromatin appears finely granular and the nuclear membrane is not sharply defined, the second is undergoing a process of degeneration and fragmentation, while the third is developing normally. The nucleus has lost its finely granular aspect and has become more or less opaque with a few ill-defined darker spots. A ring of cytoplasm has condensed about it (mitochondria) in which dark granules are scattered. In the next stage (Fig. P, 4) a portion of the ring has differentiated into a tail, between which and the nucleus two large dark spheres have arisen. Down the center of the tail also runs a slender dark filament. In the mature spermatozoon usually but one of these spheres is discoverable. In pole view (Fig. P, 6b) rays are seen to extend from it to the nucleus. The nucleus is now lens-shaped, sometimes cupshaped. (Fig. P, 5, 6a.)

With regard to the homologies of this spermatozoon as compared with others of the same order, the dark sphere is no doubt the centrosome and the tail filament its derivative. The region in which the sphere lies is therefore the middle-piece and the striations may be compared to those visible in the spermatid of Hippa. (Fig. Hi, 24.) This spermatozoon differs from the others inasmuch as the middle-piece is not so well marked and part of the cytoplasm remains surrounding and anterior to the nucleus.

Fig. P. 7 represents an abnormal spermatid in which the nucleus has failed to condense, while the centrosomes are broken into granules.

The spermatozoa of the Isopoda are characterized by their aggregation into bundles bound together in a common sheath. Distinctness of cell boundaries is very early lost between the young spermatids. In Oniscus I have sometimes seen in this syncytial protoplasm bodies staining more or less darkly and showing some tendency to form threads. It seems quite possible that they corespond to the mitochondria of the Decapoda and that the long threads which soon arise in the syncytial protoplasm originate from them. In Oniscus I was unable to make out middle-piece and centrosome, but in Idotea they may occasionally be seen (Pl. 2. I, 22-25), although it is not apparent that the middle-piece arises from any previously present mitochondrial body.

The behavior of the nucleus is somewhat different in the two forms. In Idotea the chromatin very early leaves the center of the nucleus and is massed in irregular clumps at its periphery. (Figs. I, 19-20.) The irregular masses next merge together at one pole, while the rest of the nucleus becomes attenuated. (Pl. 2. I, 21-22.) The cap-shaped nuclear mass, as it elongates, encloses this attenuated portion of the nucleus, which is finally reduced to a mere vacuole or breaks up into a number of smaller vacuoles distributed through the substance of the nucleus. (Figs. I, 23-25.) In Oniscus on the other hand the chromatin is at no time massed around the periphery, but is transformed into a network of great delicacy and without vacuoles. Probably this network holds in its meshes a more fluid substance which gradually so changes its character as to stain more and more deeply. Although in both Oniscus and Idotea the nucleus

during the median stages of its development loses considerably in staining power, it never becomes so pale as in the spermatid of Hippa. (Pl. 2. O, 4-5.)

In the young spermatid of the Amphipod Talorchestia a small, spherical "Nebenkern" may be seen. This enlarges and elongates as the rest of the cytoplasm diminishes. Finally a differentiation is brought about into middle-piece and tail. (Pl. 2. T, 15-18.) I have no stages showing a temporary loss of staining power in the nucleus, and do not, therefore, know whether this occurs in Talorchestia, or whether the nucleus shown in Fig. 15 is a direct transformation of the daughter nucleus of the second spermatocytic division.

Spermatogenesis up to the Formation of the Spermatids.

Spermatogonia.—The spermatogonia of the different forms are sufficiently unlike to permit of their being recognized without difficulty. The unlikeness is referable chiefly to the size of the nucleus itself, to the number, size, position and shape of the nucleoli, and to the manner in which the chromosomes arise.

The spermatogonia of the Isopoda resemble each other more closely than they do that of the Amphipod; also the spermatogonia of the Decapoda bear more resemblance to each other than they do to those of the Amphipoda and Isopoda. Again those of Homarus and Astacus are more like each other than like Hippa.

In Talorchestia the nucleolus is found close to the nuclear membrane and is often irregular in shape. More than one is frequently present. (Pl. 1. T, 1.) The nucleolus of the Isopoda has usually a slightly eccentric location and in Idotea is considerably larger than in Oniscus, although the nucleus as a whole is larger in Oniscus. (Figs. I, 1-2, O 1.) In the Decapoda the nucleolus is usually central or nearly so, and in all it consists of two substances staining in different degrees of intensity with iron hæmatoxylin. In Astacus and Homarus the more deeply staining substance collects around a few centres, but in Hippa it is irregularly distributed through the nucleolus. (Pl. 1. Hi, 1; Ho, 1; A, 1.) Of course the amount of granular material varies in different nucleoli and appears more abundant in darkly stained preparations.

As to the manner in which the chromosomes arise, the Decapoda and Talorchestia follow one method, the Isopoda another. In the former no spireme is found, nor even separate elongated threads; instead prochromosomal areas appear in the nucleus and the network becomes less clear and regular. The prochromosomes gradually increase in size until they are fully developed chromosomes. (Pl. 1. T, 2-3; Hi, 1-4; A, 1-2; Ho, 1-2.) The chromosomes of the Isopoda, on the other hand, arise as more or less elongated threads, a coarsening and breaking up the network as it were, which gradually shorten and condense to form mature chromosomes. Those of Idotea are generally crescent-shaped and are from the first somewhat shorter and more compact than those of Oniscus. (Figs. I, 2-3; O, 1-2.) The latter difference persists even in mitosis. (I, 4; O, 2.) The centrosome of Idotea appears to arise within the nucleus and that of Oniscus outside it, and correlated with this is a greater tendency towards polarity in the spermatogonia of Idotea, the bends of the horseshoe-shaped chromosomes pointing towards the center of the cell (Fig. I, 2.)

Synapsis.—Some difference is noticeable in the mode of transition from the last spermatogonia to the synapsis. In Idotea, Hippa and Homarus there is evidence of an approach of chromosomes in parallel pairs. (Figs. I, 5-6; Hi, 6-7; Ho, 4.) In Hippa and Homarus this is not at first accompanied by a recession of cytoplasm from the nuclear wall as is the case with Idotea. Gradually, however, the cytoplasmic connections become fewer and the chromosomes more closely associated. Those of Homarus and Astacus crowd together at one side of the nucleus, but I have not observed this condition in Hippa. (Pl. 1. Ho, 5; A, 3.) There the chromosomes mass together near the center of the cell for a very short period and then scatter more or less. During this time the members of each pair come into intimate union. Fig. Hi, 8 represents this union, Figs. 9 and 10 the immediately succeeding stages in which the members of each pair commence and continue to diverge. It is probable that a longitudinal splitting of the threads occurs at this time, although it is not always evident. The chromosomes of Idotea first appear as parallel and elongated thickenings of the nuclear network.

As the network contracts about the prominent nucleolus the ends of the chromosomes may be seen projecting from its border in pairs. (Figs. I, 5-6.) Later they shorten and approach each other more closely.

In Talorchestia, on the other hand, I was not able to make out with distinctness any parallel pairing of chromosomes. The first premonition of synapsis is the appearance of long, slightly thickened strands in the nucleoplasm converging towards the nucleolus. They gradually grow denser, but for some time retain their connection with the nuclear wall, while the rest of the nucleus condenses about the nucleolus. (Pl. 1. T, 5-7.) Finally, however, the entire network is converted into V-shaped chromosomes with the exception of a few delicate fibrils still stretching across to the nuclear membrane. (Fig. T, 8.)

The nucleolus of Talorchestia and Idotea is apparently more of a factor in the synaptic changes than that of Homarus or Hippa. The nucleolus of Talorchestia possesses considerable plasticity. Its shape is not usually truly spherical; it may be ovoid, more or less elongated or sometimes drawn out into processes not unlike the pseudopodia of an amœba. It seems to form a center around which the nuclear network contracts. In destained preparations its substance may show the color in three degrees of intensity, indicating that it is not by any means homogeneous. The nucleus of Hippa and Homarus persists throughout the synaptic changes, but is to all appearances independent of the chromatin. (Pl. 1. Hi, 11; Ho, 6.)

Maturation Divisions.—The nuclear network of the resting spermatocyte is in all delicate and stains only faintly. The condition of the nucleolus varies in the different forms. In Oniscus it is not very conspicuous, in Idotea much more so and always flattened against the nuclear membrane. In Hippa it is very small and there are frequently two. In Homarus it is rather prominent and occupies approximately the center of the cell. (Pl. 1. Hi, 14; Ho, 7; Pl. 2. I, 11.) In all it diminishes in prominence during the prophases and finally disappears.

A stronger tendency to polarity in the formation of the spermatocytic chromosomes is evident in Hippa than in the others, although

it is fairly well-marked in Idotea and also in Astacus (I, 12; Hi, 15-16; A, 4). It does not occur at all in Oniscus. Correlated with this tendency to polarity is the appearance near the center of the cell of a faintly staining body, presumably kinoplasmic in nature as it sometimes shows radiations and at other times minute granules may be seen within it. (I, 12, 13, 16; Hi, 17, 19.) In such cells also, as the chromosomes shorten and thicken, they come to lie close to the nuclear membrane, a comparatively clear space being left in the center. When the centrosomes do not arise within the nucleus, but in the cytoplasm as in Oniscus, the chromosomes remain distributed throughout the nucleus. (Pl. 2. O, 3.)

With regard to the manner of formation of the chromosomes, considerable similarity is noticeable between members of the same order. Thus in Idotea, as in Oniscus (Nichols, 1902), the components of each bivalent chromosome occupy with reference to each other one of three positions, end to end, side by side, or a position intermediate between these giving rise to the form of a crescent. A shape not infrequent in Idotea, but not noticed in Oniscus, is that shown in Figs. I, 13-14, c. Here the components are joined end to end and at their place of junction spread out slightly so as to form an incipient cross. This shape is not uncommon in insects and some other forms. Chromosomes like b in Figs. I, 13-15, clearly produced by the parallel approach of their components, may form either rings or crescents according to the closeness of the approximation. The chromosomes are more numerous than in Oniscus, it being possible to count as many as twenty-eight in the monaster stage (Pl. 2. I, 17), whereas in Oniscus there are but sixteen (Nichols, 1902). It is correspondingly more difficult to determine how many of each type are present, but there are without doubt at least as many as three of the parallel conjugating type. (Figs. I, 14, 17.) A side view of the metaphase gives much the same appearance as in Oniscus and the difference between the types is quite evident. (Fig. I, 18.) No very marked difference in size of the chromosomes can be observed in either Oniscus or Idotea, although they are not quite so nearly uniform in size as those of Talorchestia. In the latter animal they appear to be all of one type, formed by an end to end union; no rings

nor crescents are to be seen. Their number in the spermatocytes of Talorchestia is eighteen. (Pl. 2. T, 10; T, 13.)

The chromosomes of Hippa, on the other hand, are formed not by an end to end union of their components, but by their parallel approach. (Pl. 2. Hi, 17.) They are much more numerous than in the lower orders. I have counted sixty in the monaster of Hippa. (Fig. Hi, 20.) A difference in size is also a little more evident, although I have not attempted to arrange them into definite groups according to size, nor to count the number in each group.

There is always, during the prophases of maturation, an increase of cytoplasm in proportion to the nucleus. This takes place to a much greater extent in the Decapoda than in the lower orders (Hi, 14-18; Ho, 4-5), and is accompanied by the appearance in the cytoplasm of a "Nebenkern." It is no doubt correlated with the formation of mitochondria and the tail capsule of the spermatid. In the spermatocytes of Homarus and of Astacus the mitochondrial material is particularly noticeable, forming as it does dense granules in the cytoplasm. (Figs. Ho, 5; A, 3.) The increase of cytoplasm in Astacus is accompanied by a marked growth in size of the nucleus. This can be observed as early as the synapsis and throughout the maturation stages the cells of Astacus are nearly twice as large as those of Homarus.

The Male Reproductive Organs.

The testis of Idotea, like that of the terrestrial Isopoda consists of three lobes emptying separately into the vas. The testes are also alike inasmuch as within the lobes the developing germ cells do not present a continuous series but occupy zones rather sharply marked off from each other. Pl. 3. I, 28. In Oniscus and Porcellio the zones follow each other in linear fashion (Nichols, 1902), while in Idotea the arrangement is lateral.

The upper portion of the vas of the Isopoda is expanded. In the terrestrial forms it is appreciably wider than the lower portion and lined with large secreting cells. In Idotea the difference in diameter is not so great and ordinarily the cells lining the two portions do not differ much in appearance. At times, however, a few cells on

one side of the vas become greatly enlarged, and the nuclei amœboid. The nucleus at first contains granules, a few sharply staining, the numerous remainder less densely colored. It expands and fragments and the contents become finely granular, except for a few which remain somewhat larger and darker. (Pl. 3. I, 26-27.) Granules, however, do not appear in the cytoplasm, which becomes more and more vacuolated and finally commences to disintegrate. The appearance of these secretory cells is quite different from that of the land Isopoda. (Pl. 3. O, 6.) There they are far larger, the nuclear granules more nearly uniform in size, and the cytoplasm filled with similar granules which stain less intensely than those within the nucleus. (Nichols, 1902.)

The structure of the testis of Talorchestia is somewhat simpler than that of the Isopoda. It consists of a single follicle in which the germ cells develop in laterally arranged areas. There is no constriction between the testis and the upper portion of the vas and in longitudinal section germ cells are found on one side of the lumen, secreting cells on the other. (Pl. 3. T, 23.) The secreting cells are different in appearance from those of the Isopoda. Some of the cells contain nuclei comparatively small with the chromatin showing a decided tendency to gather in lumps close to the nuclear wall. (Pl. 3. T, 19.) The nucleus increases in size and sometimes becomes amœboid at its margin (Pl. 3. T, 20) and the chromosome granules become more distributed through the nucleoplasm. Other cells contain nuclei of greater size in which the chromatin is no longer applied to the nuclear wall, but consists of irregular masses connected with each other and with the well-defined nuclear membrane by a delicate network. (Pl. 3. T, 21.) In one cell a large vacuole was seen in the center. (Pl. 3. T, 22.) This condition is probably degenerative, since the cells adjacent to the germ cells disintegrate. (Pl. 3. T, 23.)

The testis of the Decopoda is more complicated in structure than that of the Amphipoda and Isopoda. It consists of numerous closely crowded follicles opening into a much coiled tube. Different follicles of Hippa contain during the breeding season different stages in the development of the germ cells and a continuous series is therefore

not difficult to obtain. The follicles of Astacus and Homarus, on the other hand, present comparatively few stages at any one time.

Summary and Conclusions.

The foregoing review and comparison shows that within the limits of the Class Crustacea differentiation of the chromatin occurs to a degree appreciable to our vision and in a way that is to a certain extent characteristic of the orders included in the class. The differentiation takes place as a divergence of form and relative size.

Thus in the Amphipod little difference is noticeable in the size or shape of the chromosones, but in the Isopoda two types are discoverable in the spermatocytes, those in which the components are joined end to end forming rods or dumb-bells and those in which they are joined side by side forming rings, these differences being accompanied by a somewhat more marked difference in size. The chromosomes of the Decapoda, though differing from each other but little in shape, show some difference in size. On the whole, however, the Crustacea as compared with that other large class of the Arthropoda, the Hexapoda, exhibit less differentiation in the form and size of the chromosomes, nothing comparable to the accessory chromosomes having been found and none exceptionally large such as McClung has described for the Orthoptera.

As regards the number of chromosomes but little relation is evident to the degree of development. In the lower forms they may be very numerous, eighty-four (reduced number) in Artemia as described by Brauer (1893) or comparatively few, eleven or twelve in Cyclops (Ruckert, 1894). In the Decapoda they are likely to be numerous, sixty in Hippa, fifty-eight in Astacus (Prowazek, 1902), while in the Isopoda and Amphipoda there are not so many, Oniscus sixteen, Idotea twenty-eight, Talorchestia eighteen. Braun, however (1907), writing about the specific chromosome number of the genus Cyclops, draws a parallel between the reduction in number of chromosomes (from twenty-two in C. strenuus to six C. gracilis) and a gradual reduction in the development of the rudimentary swimmeret. According to his account also, in C. viridis two of the chromosomes are much smaller than the others and in C. prasenus one is smaller than the others.

The formation of the chromosomes from the nuclear network occurs in one of two ways, either around prochromosomal centers (Homarus, Hippa, Astacus, Talorchestia), or as more or less elongated loops or threads. (Oniscus, Idotea.) Of course these need not be considered as entirely distinct methods of formation. Transitions might readily occur between a condition in which there is an early determination of chromatic material towards circumscribed areas and one in which the network first breaks up into loops or strands and is later condensed.

The parallel conjugation of chromosomes during synapsis seen in Hippa, Homarus and Idotea,[1] and their subsequent divergence recalls similar phenomena described for such widely different organisms as flowering plants (Bergh, 1904), Nematoda (Marcus, 1906), Hexapoda (Otte, 1906; Stevens, 1906; Henderson, 1907) and Amphibia (Montogomery, 1903). Multiplication of such instances indicates that this may be the usual method of conjugation during synapsis, although in many cases obscured by the dense massing of the chromatin at this stage. This method of conjugation of course suggests a more intimate union of male and female components and an attraction between the elements of the chromosomes rather than between the chromosomes as a whole.

The tendency to polarity in the arrangement of the chromatin seen in Idotea, Hippa and Astacus evidently depends on the location of the kinoplasm, whether within or without the nucleus, and as the polarity is accompanied by a centrifugal movement of the chromosomes as mitosis approaches, it is a tempting hypothesis to suppose that at this period some substance is elaborated in the kinoplasmic region which sets up an outward current and that this current carries the chromosomes with it.

Relationship clearly does not determine the location of kinoplasm, for it arises within the nucleus in Idotea and without in Oniscus. To a slight degree it seems to determine the appearance and behavior of the nucleoli, but not nearly to the same extent that it affects the origin and arrangement of mitochondria.

[1]Something similar has been observed for Cyclops strenuus by Lerat (1905).

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EXPLANATION OF FIGURES.

Camera drawings at the level of the table with Zeiss homogeneous immersion objective 1/12, ocular 12.

Part of the material was stained in Hermann's fluid, the remainder in Flemming's fluid. It was stained either with iron hæmatoxylin or with safranin and malachite green.

PLATE I.

Talorchestia longicornis.

T, 1. Resting spermatogonia.

T, 2 and T, 3. Spermatogonial prophases.

T, 4. Metaphase.

T, 5-9. Synapsis.

Idotea irrorata.

I, 1. Resting spermatogonium.

I, 2 and I, 3. Spermatogonial prophases.

I, 4. Metaphase.

I, 5. Early synapsis. Parallel strands appear in the network.

I, 6. Later synapsis.

I, 7-9. Divergence of conjugated chromosomes.

Oniscus asellus Linn.

O, 1. Spermatogonial prophase.

O, 2. Anaphase.

Hippa talpoides.

Hi, 1. Resting spermatogonium.

Hi, 2-4. Prophases.

Hi, 5. Metaphase.

Hi, 6 and Hi, 7. Nuclei of the last generation of spermatogonia. Approach of chromosomes in pairs.

Hi, 8-12. Post-synapsis. Gradual divergence of conjugated chromosomes.

Hi, 13. Final conversion of chromosomes into the network of the resting spermatocyte.

Hi, 14. Resting spermatocyte. Nucleoli reduced in size.

Astacus fluviatilis.

A, 1. Early spermatogonial prophase.

A, 2. Late prophase.

A, 3. Synapsis. Mitochondria in the cytoplasm.

Homarus vulgaris.

Ho, 1. Resting spermatogonium.

Ho, 2. Early spermatogonial prophase.

Ho, 3. Very late prophase.

Ho, 4. Spermatogonium of the last generation. Approach of the chromosomes in pairs.

Ho, 5. Synapsis. Longitudinal conjugation of chromosomes. Mitochondria in the cytoplasm.

Ho. 6. Divergence of the pairs.

Ho, 7. Resting spermatocyte. Mitochondria in the cytoplasm.


T1
T2
T3
T4
T5
T6
T7
T8
T9
I1
I2
I3
O1
O2
I4
I5
I7
Hi.1
Hi.3
Hi.5
Hi.10
I6
I8
I9
Hi.4
Hi.8
Hi.2
Hi.6
Hi.7
Hi.9
Hi.11
Hi.12
Hi.13
Ho.1
Ho.2
Ho.3
Ho.5
Ho.7
A3
A1
A2
Ho 4
Ho.6

Plate II.

Talorchestia longicornis.

T, 10. Spermatocytic prophase. 18 chromosomes.

T, 11. Spermatocytic prophase. Origin of kinoplasm within the nucleus.

T, 12. Spermatocytic prophase. Centrosomes within the nucleus.

T, 13. Formation of equatorial plate. Pole view. 18 chromosomes.

T, 14. Metaphase.

T, 15. Young spermatid.

T, 16-18. Gradual differentiation of head, middle-piece and tail.

Idotea irrorata.

I, 10. Post-synapsis. Peculiar appearance of the nucleolus.

I, 11. Resting spermatocyte or very early prophase.

I, 12. Spermatocytic prophase. Origin of kinoplasm within the nucleus.

I, 13-15. Spermatocytic prophases. The chromosomes are joined either end to end (a) or side by side (b). c, cross-shaped chromosomes. 14-15, surface views.

I, 16. Later prophase. One of the chromosomes is abnormally late in condensing. Centrosomes visible within the kinoplasm.

I, 17. Equatorial plate. Pole view. 28 chromosomes.

I, 18. Equatorial plate. Side view. Two types of chromosomes.

I, 19-25. Development of the spermatid.

Oniscus asellus Linn.

O, 3. Spermatocytic prophase. Origin of centrosomes without the nucleus. Compare Figs. I, 12, 13 and 16.

O, 4-5. Young spermatids.

Hippa talpoides.

Hi, 15-16. Spermatocytic prophases. Longitudinal splitting of the chromatin. Increase of cytoplasm.

Hi, 17. Later prophase. Origin of kinoplasm within the nucleus.

Hi, 18. Later prophase. Chromosomes mostly U or V-shaped. Mitochondria in the cytoplasm.

Hi, 19. Late prophase of the first spermatocyte. Kinoplasm within the nucleus.

Hi, 20. Metaphase. Pole view. 60 chromosomes.

Hi 21. Metaphase. Side view.

Astacus fluviatilis.

A, 4. Prophase of the first spermatocyte. Polarity of chromatic loops. Mitochondria in the cytoplasm.

A, 5. Spermatocytic metaphase.

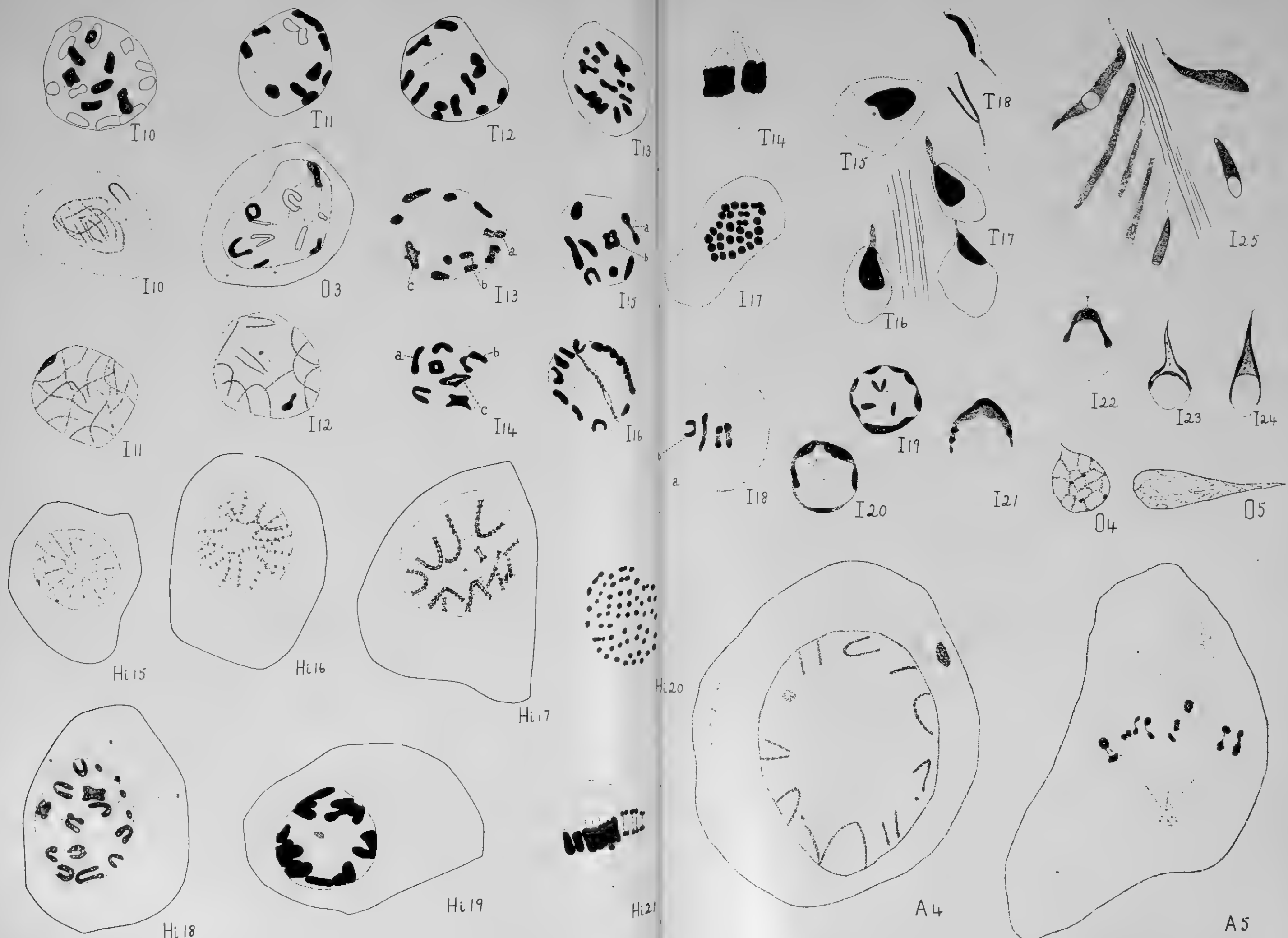

T10
T11
T12
T13
T14
T15
T18
I25
I10
O3
I13
I15
I17
T17
T16
I11
I12
I14
I16
I18
I19
I20
I21
I22
I23
I24
O4
O5
Hi15
Hi16
Hi17
Hi20
Hi18
Hi19
Hi21
A4
A5

Plate III.

Hippa talpoides.

Hi, 22. Young spermatid.

Hi, 23. Abnormal spermatid.

Hi, 24. Development of mitochondrial ring and of the strands which will become the streamers of the middle-piece.

Hi, 25. (a) Differentiation of tail-capsule and decoloration of nucleus. (b) Pole view.

Hi, 26-28. Development of tail-capsule, tube, ring and plug. In these figures the streamers are not shown.

Hi, 29. Elongation of the capsule. Condensation of x^2. Appearance of striations.

Hi, 30. a, Later stage. b, c and d, variations in the appearance of x^2. e and f, cross sections in the region of the nucleus and of x^2.

Hi, 31. a, Stage very nearly the same as 29, but of somewhat different aspect. b, cross section of x^2.

Hi, 32-34. Final stages in the development of the spermatid.

Homarus vulgaris.

Ho, 8. Spermatid before the formation of the tube.

Ho, 9. (a) and (b). Cross sections showing manner of formation of tube. (c) In the region of the middle-piece, showing the centrosome and beginnings of the three streamers.

Ho, 10. Spermatid after formation of the tube.

Eupagurus longicarpus.

E, 1-5. Development of the spermatid.

Palaemonetes sp.

Development of the spermatid.

P, 1-3. Three nuclei of the syncytium. 2 is disintegrating. 3. Condensation of mitochondria around transformed nucleus.

P, 4. Formation of tail. Two centrosomes and an axial filament are evident.

P, 5. Cup-shaped nucleus and single centrosome. The tail has elongated.

P, 6. (a) Lens-shaped nucleus. Beaded axial filament. (b) Pole view.

P, 7. Abnormal spermatid.

Talorchestia longicornis.

T, 19-22. Secretory cells from the vasa deferentia.

T, 23. Testis and vas. go., spermatogonia. cy., spermatocytes.

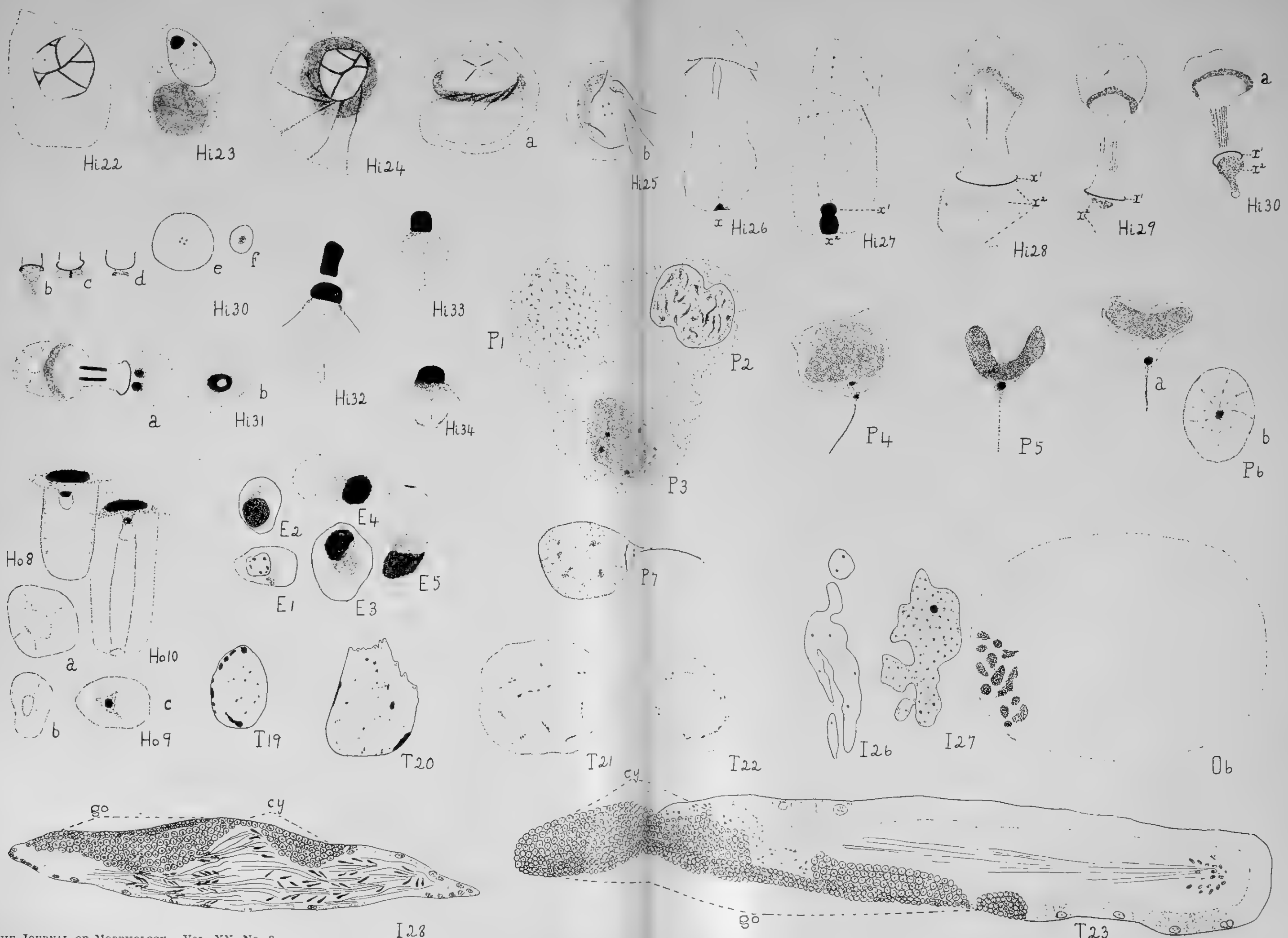
Idotea irrorata.

I, 26-27. Secretory cells from the vas.

I, 28. One of the three testis lobes, showing the lateral arrangement of the developing germ-cell.

Oniscus asellus Linn.

O, 6. Secretory cell from the vas. A small portion only of nuclear network shown.


Hi22
Hi23
Hi24
a
b
Hi25
x
Hi26
x'
x²
Hi27
x'
x²
Hi28
x'
x²
Hi29
a
x'
x²
Hi30
b
c
d
e
f
Hi30
Hi33
P1
P2
a
Hi31
b
Hi32
Hi34
P3
P4
P5
a
b
P6
Ho8
E2
E4
E5
E1
E3
a
Ho10
b
c
Ho9
T19
T20
P7
T21
T22
I26
I27
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go
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T23

A STUDY OF THE LIFE-HISTORY AND HABITS OF CHÆTOPTERUS VARIOPEDATUS, RENIER ET CLAPAREDE.

HOWARD EDWIN ENDERS.

WITH THREE PLATES.

INTRODUCTION.

While I was at Beaufort, North Carolina, during the season of 1903, I became interested in the peculiar modifications that adapt Chætopterus to its sedentary mode of life. At the suggestion of Dr. E. A. Andrews I undertook to study its embryology and anatomy the following season, but was unable to rear the larvæ beyond the stages which E. B. Wilson has described. Toward the end of the season of 1905 I was successful in rearing larvæ from eggs to such a stage that it was possible to know the younger larvæ collected in the towings. I was fortunate enough to find a sufficient number of Chætopterus larvæ in the towings to observe the important stages in the life-history and to rear the younger ones in the aquaria. In a brief time they grew to stages that correspond with the young worms on the sand flats. I have now two worms living in the laboratory of the Johns Hopkins University that grew from larvæ taken in the tow-net more than ten months ago.

The muscular movements of the older larvæ were so strong and rapid that it was impossible to make projection drawings of them. Narcotization was unsatisfactory, so they were drawn by the aid of measurements made with a micrometer eye-piece.

I have sectioned some of the larvæ, but the number of specimens is insufficient for a complete study. I hope to present the results of a histological study of Chætopterus in a later paper.

The presence of hundreds of *Spiochætopterus* larvæ, which, in the younger stages, resemble the younger larvæ of Chætopterus, confused me for two weeks until I permitted several of each genus to settle down and complete their transformation. When this was completed I discovered that the majority of the transformed individuals were Spiochætopterus.

The fertilization and segmentation of the egg has been described by Mead, and the free swimming young have been described by Wilson, therefore these subjects will be passed over briefly. My own description will have reference to the transformation and development up to the period at which the definitive characters of the adult form have begun to appear. In order to properly interpret the transformation I shall take up the adult morphology before dealing with the embryology.

In my study of the adult worms I have verified J. Joyeux-Laffuie's monograph on Chætopterus but will here describe chiefly such parts as will aid in understanding the transformation of the larva. Throughout the paper I have usually used Laffuie's nomenclature for the adult and also for the larva.

The work, of which the present paper is an account, has been carried on in the Zoological Laboratory of the Johns Hopkins University, and in the Fisheries Laboratory at Beaufort, North Carolina.

I am indebted to Honorable George M. Bowers, United States Commissioner of Fish and Fisheries, for the privilege of occupying a table in the Fisheries Laboratory at Beaufort, North Carolina, and to Dr. Caswell Grave, Director of the Laboratory, for many privileges extended. To Dr. E. A. Andrews, of the Johns Hopkins University, I am indebted for valuable suggestions in the work. I desire to express my thanks to Mr. Charles Hatsell of Beaufort, North Carolina, for specimens of living Chætopterus, which he collected and sent me during the winter of 1905.

To Professor Brooks I express my hearty appreciation of his interest and friendly counsel in the direction of my work.

Chætopterus variopedatus, Ren. et Clapd., is a widely distributed tubiculous annelid of the family Chætopteridæ. The individuals of each country and of widely distributed areas in Europe were

classified as distinct species, but Joyuex-Laffuie, '90, showed that the nine European species are really a single species. He also suggested that a close study of the six species in foreign seas would yield a similar result. Later, Græffe, '05, mentions *Chætopterus pergamentaceus,* Andouin et M. Edwards, the name by which the American species has been described by Verrill, E. B. Wilson, and others, as synonymous with *Chætopterus variopedatus.* A careful comparison of the specimens found at Beaufort, with Joyeux-Laffuie's description of *Ch. variopedatus* leads me to regard the species as synonymous.

The characters are briefly as follows:—Anterior region is composed of eleven segments of which nine are setigerous. Middle region, five segments. Posterior region, segments numerous, but variable to fifty.

Buccal funnel large, forms the anterior part of the body. Tentacles two, conical, lateral 8-12 mm. Eyes two, just lateral to base of tentacles, brownish. Nine setigerous segments are notopodia, conical, lateral and with a thickened plastron between on ventral side; setæ lance-shaped, some in fourth setigerous segment black, club-shaped, truncate. Pair of epaulet-like structures at posterior margin is neuropodia, armed with serrate uncinal plates.

Middle region.—Composed of twelfth and thirteenth segments and three palettes. Twelfth segment bears long aliform notopodia on dorsal side and adhesive disc on ventral. Thirteenth segment, the accessory feeding organ above and adhesive disc on ventral side of second and third. Dark green dilated intestine on dorsal side between twelfth segment and first palette (fourteenth segment).

Posterior region.—Segments decrease in size regularly backwards—variable from 2, 3 or 4 in young to 500 in old individuals. Each bears a pair of conical notopodia directed dorsalward, a pair of internal lobes and a pair of external lobes on the ventral side of the body. External lobes with cirrus. Anus dorsal.

Color.—Transparent yellowish-white anterior region; middle dark green about intestine; posterior, reddish-yellow in females, white in males.

Tube.—U-shaped, opaque parchment-like, white annulated ends, dirty yellow in horizontal and vertical portions, covered with grains of gray sand. Orifices diameter 1 to 7 mm. Length of the tube 18 mm. to 50 cm. Width of tube 1 mm. to 4 cm. Length of worm 1 cm. to 30 cm.

Habitat—Collecting—Morphology.

Chætopterus variopedatus, the peculiar species of sedentary annelid, upon which the following study has been made, is found in several localities in the harbor of Beaufort, North Carolina, where the conditions for its existence are afforded by the extensive sand-flats,

either covered with a thick growth of diatoms or continually exposed to currents of water heavily charged with diatoms. It is here found living within its broadly U-shaped parchment tubes in nearly every portion of the harbor wherever the sand-flats are formed in the quieter waters. It is less abundant on the tide-swept flats of coarse sand, and so far as I know it has never been taken during any of the dredgings in the channels; this may be due to the rapid changes in the shifting channels within the large but shallow harbor.

The shallow water in which the worms live here is in marked contrast with their position along the coast of France where Joyeux-Laffuie procured the animals either by dredgings in water ten meters deep or from among the masses of tubes cast upon the shore after the great storms from the north and northwest. Along the coast of France the annelids are confined to the deeper water on account of the shifting sands of the shore, but on this portion of the American coast an abundance of material may be collected with a spade at a single low tide. At Beaufort, during three summers, I found only two mutilated tubes cast upon the shore outside of the harbor after heavy storms. It is rather more likely that these tubes were carried out from the harbor than that they were torn up from the deeper regions beyond the harbor.

The presence of Chætopterus may be recognized at low tide by the two extremities of the broadly U-shaped tubes that usually protrude several centimeters above the level of the shoal (Fig. 1). The extremities of some tubes are concealed by ascidians, colonies of bryozoans, or of hydroids, attached to them so that it may be difficult to detect their circular whitish openings within the cluster of attached animals. Usually two tubes are visible above the surface of the sand, but three are frequently found, as I shall show later (Fig. 2). After both extremities, which are from fifteen to fifty centimeters apart, have been located, it is a very simple matter to remove the tube by simply raising it with a spade and then gently freeing it from the wet sand. Where the three tubes are present the third may be overlooked and the animal may be mutilated in removal.

The animals may be removed from the tubes in perfect condition by ripping them lengthwise with one's fingers. The habit of the

animal to shrink to one end of the tube makes it difficult to remove it without danger of injury when any sharp instrument is used.

When the tube is opened the animal is found attached to the inner wall by three adhesive discs. These are on the ventral side of three successive segments posterior to the region of the body which is frequently mistaken for the "head," but which is the anterior region of the body fused with an inconspicuous head.

The most noticeable features in the worm are the extreme delicacy of its tissues and the great diversity in the form of its segments. They are so much unlike those found among others of the polychæte annelids that it would seem, at first sight, to be impossible to show the homologies between the different regions. This, however, has been done by Joyeux-Laffuie.

Chætopterus, like all the other polychæte annelids, is made up of a number of segments which varies considerably with the age of the individuals. There is, withal, such a similarity among the segments in several portions of the body that they may be considered in three groups, as Joyeux-Laffuie has done. The anterior region consists of eleven segments (based by Laffuie upon the study of the nervous system) of which the last nine are setigerous segments. The middle region consists of five segments and the posterior region has from two, in the youngest individual, to fifty in the largest specimen.

The dorsal side of the body (Figs. 3, 4 and 5) may be distinguished from the ventral by the presence of the very evident ciliated groove which arises directly back of the dorsal lip of the buccal funnel and extends to the mid-dorsal line of the first segment of the middle region. It also bears a pair of tentacular cirri at the anterior end of the superior region, and large aliform appendages on the first segment of the middle region (12th segment). The fourteenth, fifteenth and sixteenth segments (3d, 4th and 5th in middle region) have the form of palettes, but each of the segments of the posterior region bears a pair of conical notopodia which are directed dorsalwards.

The ventral (Fig. 6) side is broader and more muscular in the anterior region than any other region of the animal. The ventral side of the fourth setigerous segment (6th segment) bears black club-shaped setæ, which give this region of the body the appearance of

bearing "eyes." The pair of dorsal longitudinal muscles common to many of the annelids is lacking in Chætopterus, but a pair of thick longitudinal muscles is conspicuous over the middle region. Each of the three foremost segments of this region bears a more or less shield-shaped disc. The posterior region bears a pair of external lobes of the parapodia which diminish progressively in size to the anal segment.

The anterior region.—The anterior region is firmer, somewhat more opaque and more muscular than the remaining regions of the body. Through its transparent dorsal surface are seen the septa which correspond in number with the nine conical dorsal rami on each side of this region. Its outline is better described as a tall trapezoid than as a parallelogram, as Laffuie does. I would also differ from this author in regard to the dorsal and ventral surfaces, which are convex. In cross-section this region is elliptical in outline. (Fig. 15.)

The mouth of the adult is at the forward end of the anterior region. It is surrounded ventrally by a broad flattened lip which is continued dorsalward on each side in the form of an auriculate appendage that overlaps the base of the tentacle of the same side. On the whole it forms a broad funnel which is incomplete on its dorsal side. The mouth is bounded dorsally by a thickened edge whose anterior margin may be a rectilinear line transverse to the axis of the worm, or it may at times form an obtuse angle with the auriculate appendages of the ventral lip. Its form is dependent upon the activity of the living animal, and the form assumed in the preserved specimens does not well represent it.

A pair of slender conical tentacles is located on the dorsal side of the mouth immediately back of the thickened dorsal lip. At the lateral margin of the lip the dorsally-directed auriculate lobes of the ventral lip of the buccal funnel cover the base of each tentacle. They bear a groove on their inner margin which is covered with a ciliated epithelium as Laffuie says. In Spiochætopterus the tentacles are as long as the body of the worm and the ciliated grooves function as important accessory feeding organs.

The nine setigerous appendages are conical structures arranged at the lateral margin of the anterior region. When they are extended

their length is half the width of the plastron and the diameter of the base half the length of each appendage. Together with the broad plastron they either form a plane, or the whole may be directed dorsalward at the margin so that a cross section would be crescentic in outline. The integument of the dorsal side of the appendages is more transparent than in any other portion of the anterior region and the setæ with their protractor muscles may be seen through its transparent walls. The setæ are arranged in the form of a fan. Their lanceolate ends protrude in a line along the ventral side of each of these rami and are directed laterally along the rami. The ventral setæ of the fourth setigerous segment are black and somewhat club-shaped at their external ends. Their shafts are thick and bear annulations which are not detected in the slender shafts of the lanceolate setæ. The number of setæ varies with the age of the individual. I have found as few as five in each parapodium in a young individual to ninety in an adult specimen of average size.

The first pair of setigerous appendages is located just beneath the dorsally-directed ends of the ventral lip of the mouth. It is considerably smaller than the other parapodia, which increase progressively to the ninth pair. These conical appendages are regarded by Jourdain as the dorsal rami of the parapodia, and the somewhat triangular epaulet-like appendages of the ninth pair he regards as the ventral rami of the parapodia.

The ventral surface of the anterior region consists largely of a yellowish to a reddish yellow granular area surrounded by a narrow border of lighter color. It extends around the whole ventral surface of the anterior region encircling the ventral lip and extending between the rows of dorsal rami on each side and the yellowish "plastron" just described. In section the plastron consists of long columnar cells of ectoderm (Figs. 15, 16).

The dorsal surface of the anterior region bears a ciliated groove (Figs. 5, 15-18) which extends in a middorsal line from a point just behind the thickened rim of the dorsal lip of the buccal funnel, to the middle of the first segment (twelfth of the whole animal) of the middle region. At this point it diverges to the right and to the left along the large aliform appendages. It functions as an important accessory feeding organ, as I shall show.

The "eyes" of the adult are frequently overlooked, but they were clearly pointed out in Laffuie's monograph. One black eye-spot is located on each side external to the antenna and directly beneath the dorsally directed auriculate lobe of the ventral lips of the buccal funnel. It is here less conspicuous than in the larva. The dorsal eyes of the larva are not demonstrable in the adult individuals.

The middle region.—The middle region is situated immediately back of the ninth setigerous segment, and communicates with it by a narrow cylindrical region little thicker than the ventral muscles themselves. It is conspicuous because of the diversity in the form of the segments and for the dark green color of the intestine as seen through the transparent integument which covers the dorsal side of the body.

The number of segments in this region of the body is disputed by various workers. De Quatrefages and Jourdain include the twelfth segment, with its large aliform notopodia, in the middle region, but Laffuie accepts the former view "as being the most natural." More recently (1897) Ray Lankester published a figure in which he includes the twelfth (his eleventh) segment in the anterior region, but it may have been drawn in conformity with the views of Lespes and Cosmovici. My observations upon the extent of the œsophagus and its transition into the dilated green portion of the gut, both in the adult and the transforming larva, confirm the opinions of Laffuie and Jourdain. I shall, therefore, regard the twelfth segment as a part of the middle region, which is made up of five segments. Of these the last three segments have the form of "fans" or "palettes," and consist, according to Laffuie, of the fused notopodia and neuropodia of their segments. The thirteenth segment (second of the mid-region) is usually described as consisting of a ventral and a dorsal sucker, but I shall call the "dorsal sucker" or "dorsal cupule" an accessory feeding organ for reasons that follow in another chapter.

The twelfth segment (first of the middle region) on the dorsal side bears a pair of large flattened aliform notopodia. When they are extended at right angles to the long axis of the worm their outline forms a tall isosceles triangle whose base joins the walls of the body. The distal fourth of their dorsal margin bears a small spatu-

late plane surface at right angles to the plane of flattening of the notopodia. A complicated ciliary furrow on each notopodium extends from the proximal end of the spatulate surface along the dorsal margin of the notopodium, to a mid-dorsal position where each meets the furrow from the opposite side. At this point these furrows unite with the furrow from the anterior region. The worm, when the tube is opened, is frequently found with its aliform notopodia bent obliquely forward and dorsalward till the spatulate surfaces join. The large open arch thus formed brings the ciliated furrows into the inner border of the arch where it can serve most effectively as an organ for collecting the food. This function will be referred to in a later paragraph on the feeding habits.

The aliform notopodia are strengthened internally by the presence of numerous straight, slender setæ which do not protrude to the exterior except in specimens that have begun to macerate. They are arranged parallel to one another and oblique to the long axis of the appendage. The ventral rami, or neuropodia, of the twelfth segment, are fused to form an elliptical adhesive disc which is concave ventrally. It, together with the adhesive discs on the ventral side of the next two segments, attaches the animal to the inner wall of the tube. In form and size the disc of the twelfth segment agrees with the adhesive disc of the thirteenth segment, therefore the description of one will answer for the other. The long axis of both adhesive discs is equal to or greater than the width of the plastron of the superior region and the width is equal to about half the length. They bear, along their free anterior and posterior edges, numerous brown uncinal plates with serrate edges, as has been well described by Laffuie in tracing out the homologies of the parapodia. Of the structure of these small saddle-like ventral suckers or adhesive discs Laffuie says (page 261): "The fusion is indicated by a median furrow which presents a little depression toward its middle." This is especially true for the adhesive disc of the fourteenth segment (third of the mid-region), but it is not observable in the large adhesive discs of the twelfth and thirteenth segments. Just dorsal to the adhesive discs are the two large ventral muscles. They extend along the ventral side of the mid-region from the epaulet-like neuropodia of

the eleventh segment in the anterior region through the twelfth and thirteenth segments as large parallel cylinders. The dilated green intestine rests upon their dorsal side.

The thirteenth segment (second of the mid-region) includes all that portion of the animal between the twelfth segment and the first palette. When fully extended it may be as long as the whole anterior region. In the middle of its ventral side is found the adhesive disc mentioned and described above, as setting like a small saddle over (*i. e.,* beneath) the ventral longitudinal muscles. It communicates laterally by two yellow strands of tissue, continuations of the body cavity, with Laffuie's "dorsal cupule" in such a manner as to suggest a "two-collet ring" about the worm. I shall, provisionally, name the "dorsal cupule" the *accessory feeding organ* of the second mid-segment. On the anterior half of the dorsal side is the greatly dilated light green portion of the intestine. During life it is marked here and there by purse-like swellings which are in constant rhythmic motion from before backwards. The posterior half of the dorsal side consists of the convoluted portion of the conspicuous green intestine, whose whole surface is traversed by peristaltic waves.

Upon the dorsal side and about the middle of the segment is situated the hollow muscular accessory feeding organ which was mentioned above. It has the form of a cowl or hood which opens dorsally (Figs. 2, 3, 5 and 7). Its smaller open end is joined by a narrow pedicel to the two diverging, tubular portions of the body-cavity which unite to the ventral adhesive disc and thus give to the whole the general aspect of a "two-collet ring." From the pedicel of this structure a solid strand of muscle extends anteriorly over the dorsal surface of the intestine to the base of the twelfth segment. Cosmovici's view that this was a continuation of the ciliated groove of the anterior region was plainly shown to be erroneous. When the ventral muscles contract the dorsal strand also contracts and causes the accessory feeding organ to be raised vertically or tilted forwards (Figs. 3 and 4) so that it takes a position somewhat within or immediately back of the arch which is formed by the joined aliform notopodia of the twelfth segment. The accessory feeding organ bears gland cells scattered over its outer surface, but the inner surface bears a ciliated epithelium.

Laffuie is probably correct in giving the homology of this accessory feeding organ as follows: "The dorsal sucker (cupping-glass) formed by the terminal portions of the two dorsal rami united in the median line, is rounded externally and hollowed out internally into a deep cavity. Longitudinal and transverse sections clearly show its structure. The wall is double and the space comprised between the two walls is a diverticulum of the general cavity." My observations, which are based on dissection and sections, verify his observations that the wall is double and that the space between the walls is a diverticulum of the general cavity. Numerous muscle strands join the inner and outer walls of the pouch to one another. In regard to the function of this accessory feeding organ Laffuie says (page 264): "This dorsal cupule functions as a true sucker and enables the animal to adhere to the inner wall of its tube. This is easily verified by carefully removing a few Chætopteri from their tubes. This sucker is the only part situated upon the dorsal surface which could be used for holding the animal in its tube." I never once, among more than two hundred specimens, observed the structure in use as an attaching organ, but I have frequently observed that in some individuals it contained a greenish mass of diatoms and other organic matter mingled with mucus. The presence of nutritive material in this pouch led me to search first for a direct communication with the intestine at this point in order to acount for the presence of this mass of food so far removed from the mouth. The introduction of finely divided particles of carmine in sea water into the pouch at once demonstrated a strong ciliary current running directly or obliquely from the margin to the deeper portion of the pouch. Here the particles were rotated for some time, but they were later discharged from the pouch in a manner that will be taken up in the next chapter.

Fourteenth, Fifteenth and Sixteenth Segments.—The fourteenth, fifteenth and sixteenth segments together are somewhat shorter than the anterior region, but when the animal is extended they occupy nearly half of the middle region of the body. They form a series of very muscular campanulate organs, each with its base directed forward. One side lies against the ventral longitudinal muscles from which they derive the muscle strands that make them effective

"fans." The fans exhibit a continual rhythmic movement that usually sweeps the water towards the posterior end of the animal. On account of their similarity in nearly every respect a description of one of the segments will answer for the remaining ones.

The fifteenth segment (fourth of the mid-region), when in the middle of a beat, has the form of a broad bell. Its base, which is directed toward the forward end of the body, is covered with the delicate integument of the body. At some distance from and parallel with the periphery of the bell the integuments of this base are joined to the integuments of the top (*i. e.,* posterior convex portion) by means of delicate cross-strands of muscular tissue. This produces on its anterior surface a shallow semi-circular furrow that is parallel with the edge. The cavities which are found within the bell are diverticula of the general cavity. Within the central portion is seen the greenish tinted sigmoid curve of the intestine, and in the lateral cavities are seen the vesicular portions of the third pair of nephridia. The ventral side of the "bell" rests with its whole length upon the ventral longitudinal muscles in such a way that its rim surrounds these muscles ventrally. Immediately posterior to this rim and in the middle of the segment is a pair of thick spatulate appendages which are directed posteriorly. The presence of several close-set rows of brown uncinal plates on the distal margins of these neuropodia, and an additional pair of small papillæ, which bears uncinal plates, near the ventral margin of the bell, confirms Laffuie's conclusions on the homology of these segments with the other segments of the body (page 267): "This papilla represents the external lobe of the ventral ramus and is homologous with it. It is consequently the homologue of the superior edge of the ventral suckers of the twelfth and thirteenth segments." "The central part of the palette represents the body of the animal, while the peripheral part is the homologue of the ventral rami, rami which are fused to the walls of the body by their inner face."

The fourteenth segment (foremost palette) bears a small adhesive disc of the same form as those of the twelfth and thirteenth segments. It is intermediate in structure between these and the large neuropodia just described for the palettes. That it is homologous

with the internal lobes of the neuropodia is indicated by uncinal plates at its posterior margin alone. The external lobes are represented by small papillæ on the margin of the palette.

Posterior region.—The posterior region consists of the segments between the sixteenth and the anus, which is on the dorsal side of the terminal segment. The number, which varies considerably with the age of the individuals, I have found from two, in a very young specimen about one centimeter long, to fifty in an adult about thirty-two centimeters long when extended. Since the segments are identical in form, but in size they diminish progressively to the anus, the description of a single segment (*e. g.*, the nineteenth segment) will suffice for this whole region. The course of the intestine appears as a greenish trail over the whole dorsal side.

When the animal lies in its normal position in the tube the lateral sides of the segment expose a pair of swaying conical notopodia whose distal ends are directed vertically upward or obliquely outward from the mid-dorsal line of the body. When filled with the sexual products they are opaque, at other times transparent. Toward the anal segment, however, these notopodia are directed more and more laterally till finally the last few pairs extend out horizontally and may be directed posteriorly. Through the transparent integument of the notopodia may be seen a bundle of straight slender setæ of which no portion extends to the exterior in fresh specimens. In mature males during the breeding season the cavities of the notopodia are completely filled with sperm which gives this region a milky white appearance (Fig. 4). The eggs, which float in the general cavity, and the convoluted ovary give the sexual segments of the females a roseate yellow tint. On the posterior (inferior) surface near the base of each of the notopodia, *i. e.*, between the notopodia of adjacent segments, are located the nephridiopores. Each has the form of a slight transverse slit in a small elevated area (Fig. 8).

The ventral aspect of a segment is complicated through the presence of a pair of median (internal) and a pair of lateral (external) lobes of the neuropodia (Figs. 6 and 8). The internal lobe has the form of a cylinder which is slightly flattened antero-posteriorly. It is united along a portion of its mesial side with the adjacent lobe

of the same segment. The free distal margins bear a row of uncinal plates. They have already been pointed out in the homologous structures: the posterior margins of the adhesive discs, the similar but larger spatulate neuropodia of the mid-region, and the epaulet-like appendages of the eleventh segment (last of the anterior region). The external lobes of the neuropodia are situated just lateral to the internal lobes, so that a narrow cleft exists between their bases. These lobes are larger than the internal lobes. In form they resemble a greatly thickened broad-ax whose head forms the thick pedicel (Figs. 6 and 8). Its free edge lies on the same plane with the segment but it is directed obliquely outward from a mid-ventral line. The edge bears a row of uncinal plates and has been homologized by Laffuie with the same patches of uncinal plates on the fourteenth, fifteenth and sixteenth segments, and to the anterior margins of the adhesive discs of the twelfth and thirteenth segments. The outer edge of the external lobe bears a slender conical cirrus in whose cavity the sexual products of ripe individuals may be seen by transparency. The form and the tint of the branches of the parapodia vary greatly with the turgor.

The nephridia have been mentioned only incidentally. As pointed out by Laffuie, the first pair of nephrostomes occurs in the twelfth segment (first of the mid-region) and the nephridiopores open to the exterior in the succeeding segment. Each segment back of this, except a few terminal ones, bears the nephridiopores of one pair of nephridia and the nephrostomes of the succeeding pair. The nephridia of the segments back of the sixteenth serve both as organs of excretion and for the discharge of the sexual products.

The sexually mature males and females may be distinguished with ease by the color of the sexual products which in the posterior region fill the body cavity and its diverticulæ. The males appear milky white and the females are more yellow or roseate yellow. The eggs can be seen surging about with the general movements of the body. Adults that have discharged their sexual products can still be distinguished by the same characteristic colors of the more slender convoluted testes or ovaries, but the segments are more transparent. The sex of immature or young specimens can not be distinguished by any difference in color.

The Digestive Tract.—The digestive tract of the adult Chætopterus forms a comparatively straight tube except in the middle region of the body where it forms several convolutions. Its greatest diameter is reached in the middle of the body, but in the posterior region it diminishes slightly in calibre.

The large mouth and broad buccal funnel have been described as being located at the anterior end of the anterior region. At the inner end of its dorsal lip the mouth communicates with a tubular esophagus which is usually flattened from right and left sides so that, in sections, its lateral walls lie parallel and nearly touch one another. The flattening is produced chiefly by the presence of the muscular partitions of the setigerous segments. The flattened esophagus occupies nearly the whole dorso-ventral space within the anterior portion of the anterior region (Fig. 15). Throughout the posterior portion of this region it is circular or elliptical in transverse sections. Posteriorly, and in the vicinity of the ninth setigerous segment, the esophagus becomes dilated considerably so that it becomes tubular. Here it becomes differentiated into a glandular and a ciliated epithelium (Fig. 16). The epithelium of the ventral half of the esophagus consists of tall, ciliated columnar cells whose nuclei lie at their free ends. In this respect the cells are like those in the middle and fore parts of the esophagus and the buccal funnel. In its dorsal half the epithelium of the esophagus is thrown into folds. Many of its cells are glandular. The nuclei are located toward their proximal ends. The glandular portion begins in the dorsal wall of the esophagus immediately back of the ninth setigerous segment as a small group of cells. It gradually spreads out so that it occupies the dorsal half of the esophagus (Fig. 17) at a point midway between the posterior border of the ninth setigerous segment (eleventh of the anterior region) and an imaginary line joining the bases of the notopodia of the twelfth segment (first of the middle region). Immediately anterior to the bases of these notopodia the glandular portion ends blindly as a small cylindrical sac which lies dorsal to and parallel with the esophagus (Fig. 18). Within the lumen of the glandular portion, whose length is about equal to that of the cylindrical fæcal masses discharged by the

animal, occurs a layer of mucus (?) that is stained in the sections. I shall have occasion to refer to this structure in the chapter on Habits and Physiology. The esophagus, or a continuation of it, extends through the middle portion of the segment, *i. e.*, the portion bearing the large aliform notopodia, as a convoluted tube of diminished calibre. Directly back of the aliform notopodia it lies for a few millimeters in the median plane of the animal, and its posterior end extends as an invagination into the large, dilated green portion of the intestine with which it is continuous. Sections through this region show two concentric layers of epithelium: an inner layer of ciliated cells and an outer layer of cells filled with green granules.

The conspicuous dilated green portion of the intestine is confined to the thirteenth segment. Its anterior half consists of a thin-walled dilated sac, but it becomes constricted where it passes beneath the accessory feeding organ of the thirteenth segment. In the posterior half of the segment it again becomes dilated. This darker portion forms two and one-half convolutions in a horizontal plane before it reaches the fourteenth segment (the first palette). In the palette the intestine has a smaller calibre but it is still seen by transparency as a dark green tube. In the intervals between the palettes it is considerably constricted, but it again becomes dilated within each palette where it makes a siphonal curve in a dorso-ventral plane and then passes into the posterior region of the annelid. In the posterior region it is less green in color. When the animal is extended the intestine appears as a slightly sinuous tube of gradually diminishing calibre. Its constriction and dilatations correspond with the septa and segments it traverses. It opens to the exterior by the large gaping anus located dorsally at the posterior end of the animal.

Claparede described the pigmentation of the epithelium of this middle region of the body in his *Annelides sedentaires* (1873). Its distribution has since been correctly described by Joyeux-Laffuie ('90) and by Ray Lankester (Benham's observations, '97). It is distributed in the epithelial cells of the intestine in the form of spherical granules of varying size. They are embedded in the protoplasm of some of the cells in such numbers as to give the cells an irregularly distributed green color, as seen in section, with moder-

ate magnification. The presence of large globular goblet-cells confirms Benham's observation that the elongated ciliated cells which contain the green granules are associated with gland cells.

The pigment of the intestinal wall has received different names by different workers; Claparede calls it "hepatic" pigment, and Joyeux-Laffuie designates the cells as "cellules biliaires." Lankester objects to the above names. He introduced ('97) the name "Chætopterin" for the fluorescent green pigment which is extracted from the intestinal epithelium with alcohol. A comparison of its spectrum with that of chlorophyll led him to correct an earlier statement and say that it is not "chlorophylloid" (Sach's Botany, 2d ed. 1882) but, like the green pigment, "Bonellin," of *Bonellia viridis,* a metabolic product. He says (page 454), "It is impossible to suppose, in view of the fact that Chætopterus lives in the sand in a large parchment tube, that the intestinal pigment can have any function as pigment. On the other hand, it is not unlikely that it may eventually be shown that this green fluorescent 'Chætopterin' is really representative of the biliverdin of," what the author terms, "vertebrate bile."

Lankester, from Benham's observations, describes the green granules of the intestinal cells as spherical corpuscles varying in size and embedded in the protoplasm of the epithelial cells. He says they are not dissolved by alcohol entirely, but a colorless oily-looking stroma, quite structureless and translucent, of the same shape as the original colored granule, is left in the body. Dr. G. Brandes ('98), on the other hand, regards them as vegetable organisms which live as parasites. He thinks that the "Bonellin" may be identical with the coloring matter of "Chætopterin" but that the differences may be due to some impurities. If these green granules are symbiotic algæ or algæ in the palmella stage, it should be possible to demonstrate it by means of suitable nutrient solutions, as was done by Famintzin ('90) and Beyerinck ('90) for symbiosis in *Hydra viridis.*

A conspicuous ciliated groove extends within the intestine along its ventral side (Fig. 19), from about the middle of the thirteenth segment (second of the mid-region) till near its posterior end. It is made of tall columnar cells, which contain fewer green granules than the cells of the dorsal and lateral epithelial walls of the intestine.

These cells bear rather stout cilia at their free ends. The ciliated intestinal groove is not mentioned by any workers.

Vascular and Nervous Systems.—On the blood system I have confirmed Laffuie's descriptions from transverse sections of adult individuals. A peribuccal vessel communicates both dorsally and ventrally with a straight tube located in the median mesenteries which suspend the esophagus. The ventral vessel is a cylindrical or a flattened tube that extends the entire length of the ventral mesentery (Figs. 15-19). I have not observed any transverse branches from the ventral vessel, nor have I studied its posterior communication. The dorsal vessel in the anterior region occupies the dorsal mesentery, but at the posterior border of the anterior region it "spreads out dorsally and laterally and loses its walls." Laffuie claims that the colored fluids injected backward into the dorsal vessel passed out of the vascular space into the body cavity.

Likewise, I verified only from sections the general arrangement of the central nervous system. It consists essentially of a double ganglionic chain which lies beneath the ventral integuments and between the ventral muscles of the middle and anterior regions (Figs. 15-19). Its ganglia, at the level of the segments, are united by delicate cross strands of nerve tissue. Each ganglion gives off a series of from four to ten lateral branches that supply the branches of the parapodia and the skin of the same side. The distribution of these lateral nerves was the basis upon which Laffuie so well worked out the homologies of the segments. Anterior to the ganglion of the twelfth segment the heretofore parallel nerve cords diverge and extend forward along the lateral margins of the ventral plastron to the base of the ventral lip of the buccal funnel. Here each bends dorsally and backward thence, in the dorsal lip, they join over the median line to complete the dorsal loop, or "cerebral band," and the "esophageal commissure" of other annelids.

In the anterior region Laffuie says there are no less than eleven pairs of ganglia. They are united mesially by long parallel fibres which cross the plastron, and externally they give off lateral fibres to the nine setigerous segments, to the eyes and to the antennæ, and thus represent "at least eleven segments" in the anterior region.

Habits and Physiology.

Generally speaking, Chætopterus remains wholly confined within its dark brown, parchment-like tube which is embedded in the sand. Although confined within a tube it is not as inactive as its condition would suggest. So long as the tube is submerged in water and the animal is undisturbed it usually keeps a more or less constant current of water traversing its tube. The water serves the double purpose of aeration and of bringing in the supply of food.

The force with which the water is discharged from the tubes of an average size worm is quite considerable. On the shoals I have frequently dropped a pipette full of sand into the incurrent end of the Chætopterus tube before the shoals were completely uncovered. The current ceased almost immediately or was weakly reversed, and, at the end of about a half minute, the animal suddenly expelled the water with such force that it carried the sand to the surface of the water thirty to forty-five centimeters deep. When a larger amount of sand was dropped into the tubes it was expelled after a minute or more. Under these conditions the animal reversed itself in the tube as will be described in a succeeding paragraph.

Through its whole life Chætopterus lives within the same tube or enlargements of its tube, but I cannot agree with Laffuie that *"il ne se montre jamais a l'exterieur de son tube."* I have frequently seen several segments of the distal end of the worms protruded from the tubes in my aquaria and on the shoals. Two adult specimens that I collected during the summer of 1905 gave evidence, by their regenerating posterior region (in one, all segments back of the third; in the other, a large individual, all the segments back of the fifteenth), that they had protruded a portion of their body at an unfortunate moment. A portion of the "tail" is sometimes exposed above the orifice of the tube when a large quantity of sand, or other irritating matter that has been carried into it, is partly swept and partly pushed out. I have several times seen five or more segments pushed out of the tube and then again withdrawn. This was observed on days when the sand of the exposed shoal had become excessively heated near midday.

While it is quite common to see the buccal funnel just touch the

periphery of the tube or to protrude slightly from a lateral rent which is formed during the enlargement of the tube, it is rarely seen protruding beyond the orifice. Nevertheless, during a rising tide after an unusually long exposure of the shoals near mid-day one worm protruded the whole anterior region beyond the orifice and again withdrew into the interior. This seems to have represented an effort to avoid the warm water which remained in the tube. A single adult specimen which I collected during the same season had begun to regenerate the portion before the fourth setigerous segment, the one which bears club-shaped black setæ. A new mouth had formed, but it lacked the characteristic thick lips which form quite early in the larva of Chætopterus. Probably the protruded portions of the worm were bitten off by some passing fish. When these annelids are removed from their tube they may be kept several days in well aerated water without any sign of forming a new tube or any portion of a tube. After they were exposed two days outside of their tubes they began to macerate and soon died even if kept in well aerated water, but several specimens which were transferred to the broadly U-shaped glass tubes in an aquarium of running sea water were kept alive nearly three weeks.

I have reason to believe that the individuals which are kept in the glass tubes continue their normal bodily movements and behave, in general, as they would in their own parchment tubes. The glass U-tubes therefore serve as convenient receptacles for making continuous observations on their habits.

When Chætopterus is placed in a broad U-shaped glass tube about the same size as the parchment tube in which the animal was found, it takes a position in its horizontal portion. Here it lies on its ventral side with the adhesive discs of the twelfth, thirteenth and fourteenth segments attached to the wall of the tube. The notopodia sway back and forth in the water while the neuropodia are in constant rhythmic motion backwards. The distal ends of the notopodia of the twelfth segment are usually joined over the dorsal side, and the thirteenth segment is contracted so that the *accessory feeding-organ* which it bears is tilted forwards (Figs. 3, 4) till it lies within or immediately back of the arch formed by the union of the noto-

podia. The three palettes are in rhythmic motion so long as the animal is undisturbed, but when disturbed, as by the addition of carmine or masses of sand, the motion ceases for a time or its direction may even be reversed. Under normal conditions the palettes cause the water to flow over the animal from the anterior to the posterior end. The incurrent end of the tube is therefore nearest the mouth of the animal and the excurrent orifice is nearest the posterior end.

When finely divided carmine in water is permitted to enter at the incurrent end of the tube it is seen that some of its larger particles touch the ventral lip of the buccal funnel and there remain imbedded in the layer of mucus which is extruded. Others pass in the current of water over the dorsal side of the anterior region, thence through the arch formed by the joined notopodia of the twelfth segment; they are then swept around or over the posterior region, and expelled from the excurrent opening. When large granules of carmine or masses of sand are dropped into the incurrent opening the palettes beat only feebly or cause a weak reverse current, as stated above. This response occurs when the sand touches the ventral lip of the funnel or the tentacles. The animal then shrinks backwards in the tube and the palettes vibrate forwards with such energy that the irritating material is expelled to a distance of several centimeters above the "incurrent" end.

When a larger amount of sand and diatoms was added the method of expelling it varied somewhat from that just described. The animal moved slowly forwards toward the incurrent tube. Here it contracted the anterior region and caused its margin to roll dorsalwards so that it took the form of a cylinder. The anterior margin of the buccal funnel and the tips of the tentacles were pushed a few millimeters beyond the periphery of this end of the tube. The body was drawn forwards till it passed the mass of sand. The palettes of the middle region and the neuropodia of the posterior region then began to vibrate rapidly while, at the same time, the body of the worm lengthened backwards. The animal expelled the sand and diatoms, partly by means of the strong current which was produced by the palettes, and in part by the rhythmic movement of

the neuropodia. The neuropodia transfer the foreign matter backwards from one pair to the next and thence, by extension of the body, push it to the exterior. During this process ten or more of the posterior segments are pushed beyond the orifice of the tube. When the foreign matter is not expelled on the first trial the movements are immediately repeated. After all of the irritating material is expelled the worm takes its normal position in the horizontal portion of its tube and maintains a continuous current of water as it did before the diatoms were added.

Chætopterus may reverse its position in the tube. This is done while the animal is in the horizontal portion of its tube. The ventral lip of the buccal funnel and all the segments back of it successively bend ventralwards and then the whole body moves as though over a pulley situated at this point until the animal is reversed. This would bring the ventral surface of the animal uppermost if it were not righted by a twisting movement of the anterior region as soon as it has been turned under. All the remaining portions of the body are then righted in natural order. When the animal has reversed its position, the sand, which is then beyond its posterior end, is expelled from the tube as has been described above.

The reversal of the current of water follows the reversal in the position of the worm. I have seen this reversal occur twice within an hour in the glass tubes of my aquaria. It is done in from ten to twenty seconds. It may be induced repeatedly by introducing a large amount of sand into the incurrent orifice of the tube.

The animal generally lies in the horizontal portion of its tube, but it may protrude its proximal or distal end at the orifices when it removes objectionable matter and when it repairs or rebuilds the vertical arms of its tube. In all respects, in so far as I have been able to observe, the animals on the shoals, and the two living individuals which I have had under my observation more than nine months in an aquarium, in the laboratory of the Johns Hopkins University, behave like these in the glass tubes.

Even if the uncinal plates of the internal and external lobes of the neuropodia or their modifications, are used exclusively for "holding to the wall of its parchment tube," it is clear that the animal

can move about in the glass tube with freedom. The rapid vibration of the palettes does not have the tendency of "washing the animal backwards" in spite of the fact that the uncinal plates can not "penetrate the walls" of this artificial tube.

Feeding and the Nature of the Food.—The water which the sedentary annelid causes to pass through its tube bears a large amount of organic matter, and the abundance of the fæces attests to the fact that a large amount of it is strained from the water as it passes over the regions that perform this function.

The fæces, which in an average-sized worm are pretty uniformly six to eight millimeters long and one millimeter in diameter, contain the tests of many of the diatoms which Dr. Caswell Grave ('04) has found to serve as the food of oysters in the same waters. They are principally *Melosira,* two species, *Pleurosigma, Eupodiscus,* in addition to other forms which I have not determined. There were also shells of molluscan embryos, skeletons of copepods and young crustacea of several species, but grains of sand are rarely found. It may be true that other embryos have served as food for Chætopterus, but there is no trace in the fæces because of their complete digestion. The presence of eggs of Chætopterus, in sections of the esophagus, leads me to believe that animal cells, when available, likewise serve as food in these annelids.

The general ciliary covering of the large buccal funnel, the grooves of the dorsal surface and the accessory feeding organ of the thirteenth segment of the annelid serve as organs for the prehension of food in the absence of such prehensile organs as are common to many other annelids. This may be demonstrated by the application of finely divided carmine in sea-water to the ciliary grooves. When the carmine is dropped into the grooves of both aliform notopodia of the twelfth segment the small particles are swept downwards toward their bases. Here they pass from each side directly into the ciliary groove of the mid-dorsal line and are swept forwards to its anterior extremity which is directly back of the dorsal lip of the buccal funnel. They are then dropped into the mouth. To this extent my observations agree with those of Laffuie.

The particles of carmine which have traversed the ciliary groove

to its anterior end usually pass into the mouth by a peculiar muscular contraction. As they reach the anterior end of the ciliary groove the dorsal lip of the buccal funnel is drawn backwards and the ciliary groove, which now extends beyond the dorsal border of the mouth, permits the granules to fall directly upon the ventral lip of the funnel. The dorsal lip is then pushed forward and takes its normal form. Other granules pass from the anterior end of the ciliary groove laterally, along the posterior margin of the dorsal lip of the funnel. Near the mesial side of the tentacles some of the granules are swept over the dorsal lip and into the funnel, others, however, continue laterally posterior to the tentacles and the dorsally-directed auriculate lobes of the ventral lip. They are discarded at the side of the animal in shreds of mucus. Some particles of carmine which had traversed the mesial ciliary groove to its anterior end were swept to the right and left of the groove and there were swept backwards in two parallel rows to the vicinity of the twelfth segment. Here they were either discarded at the sides of the animal in shreds of mucus or were again swept into the forward current and, thence to the mouth, where they were disposed of as described before.

The particles which dropped upon the ventral lip were swept, indifferently, into the esophagus or towards the margin of the lip and discarded. The statement has been made in another part of this paper that in the accessory feeding organ of the thirteenth segment the granules of carmine are swept from its margin into the deeper portion where they are rotated and mixed with mucus. The accessory feeding organ is tilted so far forwards that it overhangs the mesial ciliary groove when the thirteenth segment, which bears it, is contracted (Figs. 3 and 4). Its muscular lips open widely and the mass of accumulated material is pressed out by contraction and flattening of the whole pouch. The carmine boluses were several times dropped on the mesial ciliary groove. They were then swept forwards to the buccal funnel, but were in each case discarded. One small mass of diatoms which was thus discarded from the accessory feeding organ was swept into the esophagus after it had traversed the mid-dorsal ciliary groove and was dropped on the strong cilia

of the buccal funnel. This has the appearance of a selective response on the part of the cilia.

The Significance of Mucus.—The presence of mucus in the ciliary currents has been mentioned. Its function in Chætopterus is probably more complicated than that ascribed to it in corals by Dr. Duerden ('06). I also found that the fine granules of carmine could not be dislodged from the surface of the animal by means of a stream from a pipette, but that they continued towards the mouth in shreds of mucus after they have been dropped upon the animal. Some granules in the mucus passed to the mouth but others were discarded. It is generally true that all of the sand is discarded at the side of the animal. Grains of sand are only occasionally found in the fæces.

The mucus aids in forming the fæcal masses in the anterior portion of the digestive tract. Whether they are formed in the short, glandular diverticulum in the dorsal wall of the esophagus (Figs. 16-18), or in the convoluted portion within the twelfth segment, I was not able to determine. The former is about the same length and diameter as the fæcal masses and it may be that the matters accumulate in this cavity and are there molded into the form of little cylinders a millimeter in diameter and six to eight millimeters long. The fæcal masses discharged by the worms in my aquarium, after the diatoms had not been agitated for twenty-four to forty-eight hours, consisted chiefly of a shell of yellowish mucus with the same form as those which contain diatoms and other organic matter. The walls of these fæcal shells bear several parallel spiral markings that make a single turn. They are probably formed when the little masses are pressed out of the dorsal diverticulum into the lumen of the esophagus. Whether the little masses are formed in the convoluted portion in the twelfth segment by constriction of its muscular wall, or in the dorsal diverticulum, is not apparent from my sections, for it must be determined in specimens killed and preserved on the shoals, because the food passes through the animal so rapidly. Throughout the whole of the intestine of the animal the food masses have the same form and structure. They are dark green, nearly black in color. In the dilated portion of the intestine they are tum-

bled about by the peristaltic movements of its walls. In the narrower portion of the intestine of a well-fed individual from four to six of the food masses lie side by side in the lumen. They are sometimes discharged from the anus singly, but more frequently by twos and threes. The ciliated groove which I described within the intestine probably aids in the movement of the food masses.

The medium in which the animals live normally is heavily charged with food in suspension. In order to make the diatoms of the aquarium available as food for the young worms I agitated the water several times daily. When the water has not been agitated for twenty-four hours or more the fæces which were discharged consisted mainly of the pellicle of mucus with only a few diatoms and the shells of young gasteropods or veligers that breed in the aquaria. An hour after the water has been thoroughly agitated the fæces which are discharged are filled with diatoms. Finely divided carmine that is permitted to enter the incurrent tubes is usually discarded from the tube in less than ten minutes as boluses of red mucus. I have repeatedly dropped these boluses into the tubes for an hour, but they were discharged in each case a few minutes after their entry. Some of the granules of carmine were ingested and then were discharged to the exterior an hour or more after their introduction and the thorough agitation of the water. The finely-divided gelatinous egg-masses of the gasteropods were also rejected in the same way and were not accepted as food.

The amount of fæcal matter discharged and the rapidity of its movements through the animal are proof of the efficiency of the ciliary grooves and the accessory feeding organ as organs of prehension. The water which passes through the tube is strained several times, as, by the cilia of the buccal funnel, the groove of the mid-dorsal side, the complicated ciliary grooves of the arch within the notopodia of the twelfth segment and the cowl-shaped accessory feeding organ of the thirteenth segment.

In the closely related sedentary annelid, *Spiochætopterus oculatus* (?) the tentacles have undergone a considerable specialization. They have the same form as those of Chætopterus, but they are as long when extended as the body of the annelid. The straight,

vertically imbedded tube does not permit a constant current of water to pass over the worm. Its pair of long slender tentacles is extended from the orifice of the tube. It scrapes the surface of the diatoms of the aquarium and those which are dislodged are swept up the ciliary grooves to the mouth, which I have never seen above the orifice of the tube. The tentacles further aid in the removal of the fæces to the exterior by means of a reversal of their ciliary vibration.

The perfection of the palettes, correlated with the U-shaped tube, more than compensates for the shorter tentacles, which, in Chætopterus are more primitive than in Spiochætopterus.

The Removal of Excreta.—The cylindrical fæcal masses are discharged into the horizontal portion of the glass U-tube where the animals lie while feeding. The masses are discharged at intervals of several minutes, but are not swept out of the tube as rapidly as they are discharged from the intestine. They remain till a fairly constant number has been discharged, then the palettes vibrate more strongly and expel them to the exterior. When the small specimens upon which I have made the observation were well fed they expelled from ten to twenty masses at intervals of four minutes.

The fæces are expelled with considerable force by the current of water which traverses the tube. Laffuie's statement that "the fæcal matters owe their density chiefly to the amount of sand in them" is true only in part, as has been stated earlier. The sand grains are usually rejected and expelled from the tube. My observations as described above refute his statement (page 310): "The interior of this tube is never soiled by the fæcal matters, which, if it had occurred, would accumulate in the steepest part and would finally obstruct it completely." "That supports the supposition that, at the moment of expulsion of the fæcal matters to the exterior, the inferior region of the animal which is occupied by the anus, ought to be situated near the orifice of the tube, in such a manner that these matters fall to the exterior."

The Form and Size of the Tubes.—The parchment-like tubes usually have the form of a broad U, which is thick-walled and wrinkled in old tubes, but thin and flabby in those recently formed. The

horizontal portion of the U is wider than the conical vertical arms that protrude a few centimeters above the substratum. The simple U-form is often modified in tubes that occur in shoals of sand and shells. The arms may here be so constructed that they turn abruptly aside from large shells that may be in their way. Tubes are frequently found with three arms protruding above the sand. These are tubes that have been enlarged by the extension of the horizontal portion and the formation of a new arm (Fig. 2). A septum at the base of the intermediate arm separates its cavity from that of the horizontal portion. I have found intermediate arms with little or no sand, some completely filled, while many have begun to macerate. Every large tube bears the shreds of one or more of these macerated intermediate arms, or the crescentic scars that mark their former union with the newly formed extension. The annulations near the orifices and the longitudinal strips of thinner, sand-covered, parchment alternating with thicker portions of the tubes will be taken up and described in the chapter dealing with the formation of the tubes.

There is great diversity in the size of the tubes. A very young worm formed a characteristic U-shaped tube three millimeters in diameter at its widest portions, and one and three-fourth millimeters at its orifices. The distance between the orifices measured fourteen and one-half millimeters, and the length of the arms (measured from the lower side of the horizontal portion as the base) was sixteen millimeters. During their breeding season I have collected specimens which ranged in length (between the orifices) from six to fifty centimeters, and with arms six to twenty-two centimeters long (vertically).

Size of Specimens Taken from the Tube.—The size of tubes, while it increases considerably with the age of the individuals, can be regarded only as a general index of the size of the annelid which it encloses. One individual which was less than two centimeters long, consisted of fifteen segments in the posterior region. It occupied a tube that measured three centimeters between its orifices. Another specimen, which had eighteen sexual segments, was found in a flabby tube that was three and one-half centimeters long; this

does not include an incompletely formed horizontal extension of two and one-half centimeters. The annelid itself was nearly five centimeters long. Likewise, among the larger tubes, one that has not been recently enlarged by a lateral extension may contain an annelid of greater length and with a larger number of segments than another tube whose length is greater as a result of such extension.

Size at Maturity.—The smallest specimen in which I found ripe sexual products was the second individual mentioned in the previous paragraph. I am unable to say whether it is a belated individual that was hatched late in the previous season, or one of the precocious individuals of the same season. However that may be, I found three specimens (No. 88, No. 89, No. 90) during the first week in September, 1905, which were about five centimeters long before the lateral extensions were made. They had twenty-five sexual segments, the foremost of which were filled with ripe sexual products as was proven by artificial fertilization of the eggs of the single female with sperm from each of the males. The tubes bore intermediate arms that contained no sand and were very recently abandoned in the formation of lateral extensions that more than doubled their length. (See photograph of No. 89, Fig. 2.) That the largest worms were found filled with ripe sexual products so early as the middle of June, makes it possible that at least these specimens could have grown from eggs and reached maturity in the same season. The development and transformation of the larvæ and the rapid growth of the young worms in aquaria, in spite of a deficient supply of food, confirms the belief that the young worms may, under favorable conditions, reach sexual maturity in the same season. Those which are developed later in the same season reach maturity during the following summer.

Laying of the Eggs.

The individuals of both sexes are found on the same shoals, and usually from one to three meters apart. The females are more abundant, and constitute sixty per cent of the individuals collected. This association of the sexes warrants the fertilization of a large proportion of the eggs as they are swept about in the currents over

the shoals. The ripe sexual products, which, as has been stated, fill the general cavity and its diverticula are extruded to the exterior through the nephridial pores of the sexual segments.

I have never been able to observe the egg-laying among the animals on the shoals, but I have observed it in a large individual that had been removed from its tube and placed in a dish of sea-water for the purpose of photographing in strong sunlight. After being in the direct sunlight fifteen or twenty minutes the water became quite warm and the animal began to extrude its eggs. Several hundred eggs had been extruded free into the water when I first observed the phenomenon, and many more were coming from the forward segments of the sexual region. They issued from the nephridial pores during the rhythmic movements of the body. These movements, which are of a peristaltic nature, may have been the means of expelling them, because they came from the pores, after each compression of the segments, in short streams one egg thick. Several thousand eggs were extruded during twenty minutes, after which the animal was killed and preserved. Transverse sections of its sexual segments include eggs in the body cavity. They may also be traced through the nephridia to their pores at the posterior (inferior) surface of the segments (Figs. 4 and 8). Although Laffuie says "the *eggs and spermatozoa accumulate in the nephridia* where they await the moment for expulsion to the exterior" (italics mine), I have been unable to confirm his statement from sections of nephridia from three of these segments, all of which contained comparatively few eggs in any portion—possibly forty to fifty in each nephridium. Dr. E. A. Andrews (Sept. 20, 1895) observed the discharge of a small number of eggs when fresh sea-water had been admitted to an individual kept in a glass U-tube and from this he concluded that they are discharged at high tide, *i. e.,* when fresh water is added to that in which they live. I have not been able to verify this nor to determine the conditions that naturally control the egg-laying; whether it occurs after a few eggs have collected in the nephridia, or whether heat and fresh water supply the necessary stimulus.

Fertilization.

Mead ('98) made the observation that the "Ripe eggs may be carried in the body-cavity several days before they are laid. During this time neither centrosome nor aster can be distinguished, though the reticulum is unusually distinct. In a few minutes after the eggs have been deposited in sea-water, however, a large number of asters are developed by rearrangement of the cytoplasmic network."

The eggs in the nephridia are to be found in the same stages as those in the body cavity and at no time do they show any sign of an entering spermatozoön or of a male pronucleus. Among the eggs discharged from the animal I found young ones whose germinal vesicles were still intact. The germinal vesicle of these young ova remained intact until the eggs were extruded into the sea-water, then it broke down and the first maturation spindle was formed. These eggs and the ones whose maturation occurred in the body cavity and nephridia may remain in this condition for an indefinite period or until fertilization occurs. The entrance of the spermatozoon, which is of the ordinary tailed form, causes the rapid formation of the polar bodies and female pronucleus, while the male pronucleus is still quite minute and at the distant portion of the egg. The egg at first is spherical and uniformly opaque, but its animal pole becomes flattened and clear at the time that the first polar bodies are formed.

Segmentation, and Formation of the Trochophore.

The segmentation was studied and correctly described by E. B. Wilson ('83) and Mead ('97). Its external appearance is, briefly, as follows. A few minutes after fertilization a delicate membrane from the oösperm and the polar bodies are extruded, at intervals of a quarter of an hour, from the clear end of the elongated egg.

In its first cleavage the oösperm is divided into two cells of unequal size, by a plane passing through the polar bodies. A large "yolk-lobe" is formed nearly opposite the polar bodies during the internal changes which accompany the first cleavage furrow. The second cleavage also is meridional and at right angles to the first. The

four-celled stage passes into the eight-celled stage by division in a horizontal plane. The lower cells are slightly larger than the upper group, but in each group one cell is larger than its neighbors. (Mead calls attention to the left-oblique cleavage in the eight-celled stage of Chætopterus). The subsequent divisions are nearly synchronous in all the cells to the thirty-two-celled stage and their size, excepting the D cells, is more nearly uniform than in the earlier stages, some cells divide precociously as there is only a theoretical sixty-four-celled stage. The embryo becomes elongated somewhat and over its whole surface soon develop cilia that do not penetrate the fertilization membrane. When the embryo is four hours of age the cilia vibrate rapidly and cause it to rotate within the membrane. Although Mead ('97) says the polar bodies are ingested by the rosette cells in the sixty-four-celled stage, I saw the polar bodies attached to the inner surface of the egg-membrane in which a larva was rotating and from which it soon escaped when the wall was ruptured. When five hours old the larva escapes from its fertilization membrane and swims actively at the surface of the water by a rotation of the body on its long axis. At this age the body is ovoid in form and is covered with cilia of uniform length excepting at the broad anterior end where there is a tuft of several longer ones. Eighteen hours after fertilization the close union of the several elongate cilia, and those of the posterior, narrower portion of the body become progressively larger backward. The body becomes more elongate and, when from twenty-one to twenty-four hours of age, the mouth opens at the ventral surface some distance in front of the mesotrochal band of cilia; and a pair of inconspicuous pigment-spots is now seen widely separated on the dorsal side of the body and forward from the mouth. When the larva is twenty-four hours old the mesotrochal band of cilia is stronger and back of it, on each side, is a stout flagellum. The mouth, which appears as a triangular slit, communicates with the alimentary canal. It consists of a transversely-directed esophagus which communicates with the dilated stomach. The stomach opens into a short intestine which is separated from it by a constriction of the walls. The anus is formed on the dorsal side of the body just in front of the posteriorly-directed,

short protuberance which is commonly referred to as the "terminal papilla."

Further Growth of the Young Trochophore.

When the young trochophore is about sixty-four hours old it is somewhat spindle-shaped. Its first mesotrochal band of cilia has begun to be atrophied and there remains to represent it only the pair of strong lateral flagella which may be seen vibrating rapidly. At the same time that the first ciliated band is undergoing atrophy a second circle is suggested by a gradual lengthening of the cilia around the region of the body within which is the intestine. Its alimentary canal practically fills all of the body cavity. The mouth has enlarged transversely and its anterior (upper) lip has the form of a lobe. It leads by a spacious ciliated osophagus into the spherical stomach which occupies the middle region of the body. The intestine is a short conical cavity separated from the stomach by a constriction or double fold of the entoderm layer. The intestine does not quite fill the perivisceral cavity posteriorly. Dorsally it opens to the exterior by the anus which is just in front of a "terminal papilla" that bears a tuft of long non-vibratile cilia. Many of the young trochophores have small pellets of diatoms in the stomach at this age.

The larvæ of six days are much like those described, and while they may be kept alive twelve or fourteen days in the aquaria do not seem to thrive. This is due to a lack of proper food in the aquaria, as I would judge from one culture of larvæ that was larger, at five and one-twelfth days, than any larvæ reared during the two previous summers. At this age they had the characters by which it was possible to connect the older larvæ which I reared in the aquaria, with those taken in the tow.

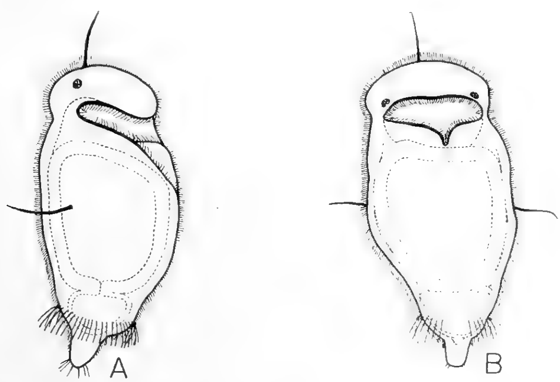
The form of the larva six days old (Text-figures A and B) is either somewhat spindle-shaped or cylindrical according as the body may be changed by muscular activity. It is somewhat longer and more transparent than in the younger specimens. It is covered with cilia which are everywhere of uniform length, excepting in the ciliated girdle around the posterior region of the larva, a pair of

lateral flagella that really consist of several parallel flagella, and an anterior flagellum. The mouth is a conspicuous large, ciliated, triangular opening surrounded by thick lips. The pre-oral lobe, or upper lip, is larger than in the younger larvæ. It has the form of an overarching hood. The post-oral lobe resembles an inverted hare-lip. From the narrow posterior end of its V-shaped cleft a groove which is provided with strong cilia, that beat towards the mouth, extends backward toward the level of the pair of lateral flagella. The brownish red "eyes" or "eye-spots" are larger and more prominent. The gullet is thick-walled and covered with close-set cilia. It passes obliquely backward and joins the spherical, thick-walled stomach at its antero-dorsal border. The intestine and "terminal papilla" are also longer than in the younger specimens. The swimming is more active and the larvæ are not confined to the surface of the water, but pass frequently to the bottom of the vessels in which they are kept. Many of the specimens of this age, as was also true of the younger ones, had masses of ingested matter in the stomach. The larvæ may, besides the rotation and forward motion, swim rapidly in a circle with the pre-oral lobe and body flexed dorsalwards. They may be kept alive in aquaria for six or seven days longer but do not develop further, in spite of one's effort to feed them on diatom cultures. To this point my observations on the development of the trochophore agree with those which E. B. Wilson made ('83) on larvæ reared from the artificially-fertilized eggs.

That the larvæ may be reared from eggs in aquaria by the use of some suitable method of providing them with animal food is suggested by the rapid growth of a single lot of larvæ during the summer of 1905. Several hundred larvæ that were two days old were transferred to an aquarium containing a luxuriant growth of diatoms (Grave's diatom method) and placed several feet from a window to avoid direct sunlight. When they were six days old the larvæ exceeded in size and development even the oldest specimens of earlier cultures. They were 1 millimeter long as compared with 0.6 millimeter for the dwarfed (and underfed) larvæ reared in earlier cultures. They formed a connecting-link between those just described and the youngest from the tow. The larvæ contained many

pellets of organic matter in the stomach and intestine. These pellets consisted of small protozoa and other low animals that were abundant in the diatom cultures to which the larvæ were transferred.

These larvæ (Fig. 9) are more elongate, and while not very different in appearance from the underfed larvæ, show some striking differences. There are three pairs of eye-spots; two pairs lateral and one pair in an antero-dorsal position just back of the anterior flagellum which still persists. The additional eye-spot on each side is smaller than, and directly in front of, the one mentioned for the previous stages. (Some individuals had, besides, a row of two or three smaller accessory pigment spots anterior to these.) The antero-dorsal pair is seen through the transparent dorsal wall of the esoph-



agus. The second band of powerful cilia which, in the earlier stage, was at the level of the middle of the intestine, is now relatively farther forward as a result of the backward growth of the stomach and elongation of the growing-region between the anus and second circle of cilia. The circle of cilia is incomplete for a short space on the ventral side as Wilson observed in larvæ which he reared from the eggs.

The ventral margin of the hemispherical pre-oral lobe has begun to be tilted forwards (anteriorly) so that the mouth now has a greater axial as well as a greater transverse diameter. The free margin of the cleft post-oral lobe is thicker and is turned ventralwards. The esophagus, stomach and intestine have not changed excepting in length.

The "terminal papilla" is an organ for attachment of the larva which, up to this time, has been pelagic. An individual two days older, which, when placed in water in a watch-glass, swam actively for several minutes, became attached by the free end of its "terminal papilla" to the bottom of the dish. With this point of attachment as a center the larva was seen to swing about in a circle or bend back and forth or from side to side, much as an attached *Stentor* does. After several minutes I saw the process released and contracted suddenly. Some individuals swam about trailing it in an extended condition as figured by Wilson (Fig. 7, Pl. 2), others used it more or less to aid as a prop in performing creeping movements on the bottom of the dish. I was unable to rear larvæ beyond this stage in the dishes, but by means of the characters which they possessed I was able to know the larvæ in the tow. The following stages are from material taken in the tow.

Beginning of the Transformation.

A Chætopterus larva 1.5 mm. long (Fig. 10) is the youngest taken in the tow net. It possesses three pairs of eye-spots and two incomplete rings of powerful cilia directly back of the middle of the body, but the apical tuft and lateral flagella are completely atrophied. The beginning of the transformation is accompanied by a change of habit. The larvæ swim slowly near the bottom of the dish instead of actively near the surface of the water, as is done by the younger ones.

The body of the larva in this stage is fusiform and bears markings on its surface that indicate corresponding regions in the adult. For this reason it will be convenient to refer to the regions as anterior, middle and posterior. The anterior region extends backward to the second (in point of development) ciliary ring, while the middle region includes the two ciliary rings together with nearly all of the remainder of the larva; the posterior region is quite small and inconspicuous at this time. The middle region is the first to become annulated in Chætopterus as it does in the very similar larvæ of the closely related genus, *Spiochætopterus,* which were more abundant than Chætopterus in the towings made in August (1905).

Anterior region.—The anterior region comprises about two-thirds of the larva. It lacks the flagella of the earlier stages and its general ciliation has begun to undergo atrophy. The mouth and the esophagus, however, retain the strong close-set cilia over their surface. The mouth has undergone further axial enlargement by a slight increase in size and further tilting of the pre-oral lobe so that its free ventral margin is nearly in an horizontal plane instead of transverse to the axis of the body. The post-oral lobe, which has increased in length by a growth at its margin, resembles a protruded tongue. The eye-spots on the pre-oral lobe are the same in number and position as in the stage just described (Fig. 10).

Nine short transverse rows of brownish-red pigment spots, on each side of the median line, mark off a shield-shaped area on the ventral side of the anterior region between the base of the post-oral lobe anteriorly and the foremost ciliary ring posteriorly. Each line of pigment-spots corresponds with the position of the setæ in the next stage.

Middle region.—The middle region is that portion of the larva which begins with the foremost ciliary ring and ends at the ill-defined constriction just anterior to the anus. It consists of two ciliary rings and three more or less clearly defined segments which decrease in size regularly toward the anus.

The ciliated rings are referred to in this paper as "first," "second," and "third" to designate their order of development. The second and third rings consist of a row of powerful cilia on a thickened ectodermal ring that surrounds the widest portion of the body, excepting for a short space on the mid-ventral side of the larva (Fig. 10).

Posterior region.—This region of the larva consists of a very short anal segment to which the growing zone is confined. Its diameter is scarcely more than the anus itself.

The "holdfast" may be regarded as a part of the anal segment from which it is a ventral outgrowth, and into which it is contracted in the transformed larvæ. When the animal is attached to a firm substance, as the bottom of a watch-glass, a considerable degree of agitation of the water is required to detach it. In this, and several

older specimens, I have seen the "holdfast" extended till it resembled a slender cord nearly as long as the body of the larva. At other times it was contracted to form a thick papilla that was marked with circular wrinkles that suggest annulation, as is shown in a paper by Fewkes.[1] The hold-fast is indifferently extended or contracted when the animal swims about in the water. It still serves as a prop by means of which the larva may push itself forwards on the bottom of the dish.

When the animal swims rapidly through the water the portion of the body posterior to the ciliated rings is contracted and flexed dorsalwards till the anus rests directly back of the third ciliated ring. The body is then somewhat pear-shaped and the rings of powerful cilia are around the thicker posterior portions. (See Text-figure

D

D of Spiochætopterus.) This position of the cilia causes the animal to swim in a wide cork-screw-like path.

Tentacles.—The tentacles appear as a small papilla on each side dorsal to the lateral angles of the mouth and posterior from the lateral eye-spots. In this stage they are covered with fine cilia.

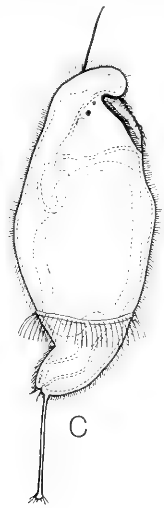
Alimentary canal.—The alimentary canal is shifted forwards and backwards by the muscular contraction of the body, nevertheless it has undergone a considerable permanent change in position.

The esophagus has become a narrow, glandular tube with muscular walls. Anteriorly it opens into the wide, funnel-like mouth, then extends into the median plane of the body to the dorsal wall of the stomach, with which it communicates about the level of the

[1]The "hold-fast" is also shown by figures in papers by Claparede and Metcznikow, M. Müller, Joh. Müller, Beranec, and Wm. Busch.

sixth or seventh pair of pigmented lines (sixth or seventh setigerous somites). The transverse septa of the setigerous somites extend towards the esophagus as they develop backward from the mouth.

The stomach is shifted backwards so that its foremost ventral wall is at the level of the fourth pair of pigmented lines (fourth setigerous segment), and its hindmost part extends through one or two segments back of the ciliated rings. In several specimens it was observed to occupy chiefly the widest portion of the body-cavity, that within the second and third ciliary rings. It is a spherical sac



C

that completely fills the region to which it is confined. Its walls are thick and have a faint tint of green at this age.

The intestine is a nearly straight tube in the mid-ventral line. It communicates with the stomach about the level of the third ciliary ring and opens at the anus which is dorsal to the hold-fast. (See text-figure C of Spiochætopterus.)

Luminosity.—The luminosity that is so striking in the adult is present in all the larvæ of this stage. It is noticeable in the vicinity of the ciliated rings, and is similarly associated with the mucus which the animals discharge into the sea-water. It is still more noticeable in the older larvæ, in the vicinity of the ciliated rings and the anterior region, when the animals are irritated.

Transformation from Free-Swimming to Creeping Larva.

The most advanced larvæ that I have collected resembled the adult worms in the form of the segments and in the transparency of the integuments. These large-bodied larvæ rarely swim about in the aquarium into which they are placed but remain chiefly at the bottom where they move among the diatoms. The parts of the body which correspond with the anterior, middle and posterior regions of the adult are marked off more clearly here than in the earlier stages. The larvæ which correspond with my figures 11, 12 and 13 were 2 millimeters long.

Anterior region.—The anterior region has shared in the growth of the larva and its general appearance has changed considerably as a result of the more rapid growth of some of its parts and the slower growth of others. Thus, its forward end has scarcely increased while the posterior portion has grown in diameter; the plastron has grown so that it is wider than the body; the pre-oral lobe has scarcely increased in size while the post-oral lobe has grown into a large spoon-shaped lip that either hangs downward over the plastron or is directed forwards and kept in the same horizontal plane as the body. This region has undergone a slight dorso-ventral flattening.

The nine setigerous segments have grown so that the foremost ones extend beyond the body of the larva. Septa have appeared between the segments and each segment bears five lance-shaped setæ which are like those of the adult. The fourth segment has not yet developed the club-shaped black setæ which are found in the adult condition.

The pre-oral lobe has relatively the same size and form, but is tilted more than in the earlier stage so that its margin now is almost in the same horizontal plane as the animal. It is covered, as in the earlier stages, with cilia that vibrate towards the esophagus.

The greatest increase in size has occurred in the post-oral lobe. It is a large lip that either hangs, apron-like, over the ventral side of the body and covers five or six of the setigerous segments (Figs. 11 and 12) when the larva is swimming, or is directed forward as in the adult worm (Fig. 13) when it is creeping. The auriculate lobes have begun to form by enlargement of its margin near the

lateral angles of the mouth (Fig. 12). They extend dorsalwards towards the eyes and bases of the tentacles when it is extended forwards. These continue to enlarge until they cover the eyes and the base of the tentacles like in the adult. The post-oral lobe is very contractile, but its lateral edge is usually curved more or less so that the concave side is turned ventralwards when the lobe is flexed, or dorsalwards when extended. This surface is covered with cilia that extend inward and are continuous with those of the gullet.

The tentacles are not so long as the pre-oral lobe. They are directed backwards, like the very much longer ones which are developed earlier in the related genus, Spiochætopterus (Text-figure D).

The five eye-spots, one of which was mesial in position, in the specimen from which Figs. 11 to 13 were drawn, suggests a fusion of the median pair. An individual of the same stage, or slightly older, had a single pair of eye-spots as in the adult. I was unable to determine whether the reduction is brought about through fusion or resorption or whether this was the number originally formed.

A shallow ciliated furrow extends, on the mid-dorsal side of the anterior region, from the anterior convex surface of the pre-oral lobe to the foremost ciliated ring. It increases rapidly in depth after the larva forms its tube, and is well developed in a transformed larva, with four sexual segments, as in any of the mature worms.

Middle region.—This region includes five segments. The foremost two bear the ciliated rings and are of greater diameter than the remaining ones which decrease in size regularly backwards. The whole region is very contractile and its segments may telescope into one another.

The foremost segment of this region, which becomes the "twelfth segment in the adult," bears an incomplete ring of powerful cilia on an ectodermal thickening of its wall. A pair of short antero-posteriorly flattened lobes at the right and left sides of the body is the rudiment of the aliform notopodia. Its position directly back of the thickened ciliary ring suggests that the ciliated groove of the notopodia is a vestige of the dorsal half of this ciliary ring, and that the notopodia are themselves formed by an excessive growth dorsalwards of the right and left sides of the rings. On its ventral side

where the ciliated ring is interrupted, are two slight mesial thickenings that represent the ventral adhesive disc of the twelfth segment. A pair of similar thickenings on each successive segment of this region indicates the beginnings either of adhesive discs or of neuropodia.

The thirteenth segment is represented in the larva by the hindmost, or third incomplete ciliary ring. On the ventral side is the rudiment of the adhesive disc as mentioned above, and in a mesial position directly back of the dorsal side of the ciliated ring is a bilobed tegumentary outgrowth (Fig. 13) that represents the accessory feeding organ of the thirteenth segment of a transformed larva. Its internal ciliation is possibly derived from a part of the ciliated ring.

The fourteenth, fifteenth and sixteenth segments are somewhat thickened, saucer-shaped discs that decrease in size regularly backwards, and have their convex sides turned forward. They develop into the palettes or "fans," in the transformed worm. Small muscle cells are present in the walls of these segments. They also form cross-strands that unite the forward wall to the rear wall, as is seen in the palettes of the worms. They are not in rhythmic motion in this stage, but are often telescoped into one another by the axial contraction of the larva. The mesial thickening on the ventral side of each segment has been mentioned: the first becomes the small adhesive disc of the first palette, and the others expand into the short, thick neuropodia of the second and third palettes.

Posterior region.—The posterior region is still very small (Figs. 11-13). It consists of two sexual segments, each of which bears a pair of divergent club-shaped to spatulate appendages that extend obliquely backward, and the rudiments of several others, in the form of buds, that are ventral to the anus. Each of the larger divergent appendages encloses a single straight seta of the same form as those found within the conical notopodia of the sexual segment of the adult worm. That these are really the notopodia of the segment, and are formed ventral to the anus but later migrate lateralwards and finally point dorsalwards, is clearly shown in a large worm that was undergoing regeneration of the segments of this region (Fig. 14). In this specimen there were nine segments in various stages of devel-

opment, from those in which the notopodia and neuropodia ranged from small papillæ on the ventral side, to notopodia and neuropodia of the usual form. In the earliest stage of its development a segment is indicated by a transverse row of four papillæ on the ventral side of the purse-like opening of the anus; forward from these the rows are progressively larger in size and more nearly like the full-grown structures of the non-mutilated region. The notopodia develop from the outer rows of papillæ, the internal lobes of the neuropodia from the middle pair of papillæ, and the external lobes of the neuropodia appear, first in the second segment from the posterior end, as small buds between the lateral and mesial rows just mentioned. The growth of these papillæ is accompanied with a lateral displacement so that the neuropodia finally occupy a position on the ventral side, and the notopodia on the lateral side of the segments. The pair of oldest notopodia of the regenerating segments, though considerably smaller than those of the uninjured part, has the same shape and is curved outward and backward in a horizontal plane that coincides with that of the body. The second and third pairs are smaller; they diverge, first ventralwards then backwards. The fourth, fifth and sixth pairs are still smaller. They are nearly parallel conical appendages on the ventral side, and between them are the small neuropodia. Back of the sixth pairs they are progressively smaller and lie wholly ventral in position.

The formation of the parapodia is the same in uninjured specimens, excepting that the contracted, or atrophied, "hold-fast" is represented by a small papilla on the ventral side between the anus and the youngest parapodial buds. The growing region is therefore perianal in position.

Digestive tract.—There has been a slight shifting and lengthening of the muscular esophagus till it now opens into the dilated, green stomach near the posterior margin of the plastron. Its walls are ciliated throughout, from the broad buccal funnel anteriorly, to its communication with the stomach.

The stomach is a large thin-walled sac within the twelfth and thirteenth segments, but which bulges forwards into the anterior region and backwards into the first palette. Its walls have a darker

green color than in the earlier stage just described but less than in the fully transformed larva. This color is undoubtedly due to the same cause as that found in the dilated green intestine of the adult *Chætopterus.* The intestine is a dilated tube within the palettes but is much narrower in the small posterior region.

Feeding.—The large size and rapid growth of the larvæ are due to their voracious habits. Nearly all the specimens collected in the towings were gorged with pellets of ingested matter. Many of these pellets contained the chitinous skeletons of small copepods, but the contents of others could not be determined because of complete digestion or the absence of skeletal structures.

In order to study their habits of feeding I put one of the oldest larvæ into a small dish of sea-water in which were several copepods, a small planarian, *Noctiluca* and diatoms. The larva moved along the bottom among the diatoms and when its post-oral lobe (ventral lip) came into contact with the planarian the mobile pre-oral lobe was pressed down upon it, the cilia vibrated rapidly and the planarian was swept into the buccal funnel. It was then rolled about and rotated several seconds by the cilia and muscular walls of the funnel till it was reduced to a mucus-coated pellet, and was then swallowed by a gulping movement of the muscular esophagus. Less than ten minutes later the same larva captured and swallowed a copepod nearly half its size. The capture was accomplished rather by the movements of the copepod than by any active attack of the Chætopterus larva. The copepod after moving among the masses of diatoms, suddenly darted to the margin of the spacious buccal funnel; it was swept into this by the cilia and was unable to escape. Another Chætopterus larva swallowed Noctiluca in the same way. Diatoms were also swept into the gullet and swallowed by the oldest larvæ. The cilia of the funnel seem to perform a tactile function, for they discard grains of sand that are taken in with the diatoms. In another instance a metamorphosing pluteus was discharged from the buccal funnel by reversal of the cilia after it had been rotated once or twice. The protruding sharp ends of the skeleton may have irritated the larva and caused the discharge.

Creeping habits.—Before this stage in its development is reached

the large-bodied larva becomes more sluggish in its movements and settles down, at first becoming attached by the holdfast. Later it creeps among the diatoms of the bottom, with its large post-oral lobe protruded forwards like a scoop. In young specimens the creeping is accomplished chiefly by the movements of the ciliated rings, and the axial contraction of the body, and, in part, by use of the holdfast as a prop; but in older larvæ chiefly by the powerful cilia of the ciliated rings and the greater contractility of the body. When they move among the diatoms of the aquaria they leave a slight trail of mucus. Later they make short, horizontal, mucus-coated tunnels into the mass of diatoms and sand. One of these tunnels may be extended to several times the length of its body and from this simple tunnel of agglutinated sand and diatoms the larva may build the U-shaped tube within which it remains confined.

Formation and Enlargement of the Tubes.

The first tube in which the larva lives and feeds for several days is nearly a millimeter in diameter and from eighteen to twenty-two millimeters long. It is either a straight tube or a shallow U whose curved portion is downward.

After an interval of a day or two in the mucus-coated tunnel the young worm, for it is now an adult in miniature, has outgrown it and a new tube is constructed. This is done by splitting the tube at a point where the upright arm meets the horizontal portion, in a U-shaped tube, or near one end, in a straight tube, and then excavating a tunnel obliquely downwards and, after nearly doubling the length of the basal portion, upwards to the surface. This is its first lateral enlargement. The sand which the worm excavates is expelled from the opposite end of its first tube. The walls of the tunnel are coated with mucus as the tunnel advances, so that the U-shaped tube is completed when the excavation reaches the surface. The tube becomes strengthened from time to time by additional layers of mucus that harden to form a parchment-like material that gives older tubes a laminated structure. They are enlarged in the same vertical plane as the original tube unless prevented from doing so by some obstruction, as a shell, when they turn obliquely along the surface

of the obstruction or construct an enlargement from the opposite end of the old tube. Two or three days later the process is repeated, possibly by an extension of the opposite end of the tube. The horizontal portion of each new enlargement is larger in diameter and is buried deeper in the sand than the tube from which it is a branch (Fig. 2). The enlargements are frequently of such length as to double the size of the U-tube, and are completed to the surface of the sand in from twenty-four to forty-eight hours. They are made indifferently at one end or other of the smaller tube. The fate of the intermediate tubes has been discussed in another part of the present paper.

The burrowing is done by the anterior region of the worm. Its setigerous segments dislodge the sand and pass it to the middle and posterior regions of the body, and they convey it backwards into the tube by the combined contraction and expansion of the body, and the rhythmic movements of the palettes and neuropodia. The worm ceases burrowing at intervals of a few minutes and expels the accumulated sand to the exterior in the same way that large quantities are removed by individuals in glass tubes (p. 505). The sand that is removed during the excavation is pushed out of one end of the tube around which it falls and forms a conical mound; the other end, or intermediate tube, is the incurrent tube so long as the burrowing is in progress, but when the new burrow is complete a septum of parchment is formed across the base of the intermediate tube and it ceases to be of any use to the worm.

The worms which form their tubes in aquaria with a thin layer of sand and diatoms on the bottom conform to the U-habit, though in a horizontal plane, with one side of the tube cemented to the floor of the vessel.

The linear extensions are formed at such intervals as the rapid growth of the worm requires. The length of the tubes, and the dates on which the enlargements were completed by two worms which I reared from larvæ taken in the tow-net, are as follows:[2]

[2]Both worms enlarged their tubes to 76 and 71 millimeters, respectively, between September 12, when they were brought to the Biological Laboratory of the Johns Hopkins University, and my return, October 4, 1905. The

SPECIMEN NO. 2.		SPECIMEN NO. 4.	
Distance between arms.	*Date.*	*Distance between arms.*	*Date.*
20 mm.	Aug. 7, 1905.	20 mm.	Aug. 7, 1905.
38 mm.	Aug. 10, "	32 mm.	Aug. 9, "
60 mm.	Aug. 15, "	61 mm.	Aug. 16, "

Early in September of 1905 I collected three worms whose tubes averaged fifty-one millimeters between the orifices, and five whose recently discarded intermediate arms were sixty millimeters from the ends with which they formed the small U-shaped tubes. The horizontal extensions increased their length to fifteen centimeters in the smallest, and twenty-two and one-half in the longest specimen. Many thick-walled tubes are found with scars of intermediate arms which indicate that they were increased from about this size to forty centimeters. The longest tubes show that they were increased, by a linear extension of ten centimeters, to fifty centimeters.

The tubes also undergo an enlargement in diameter as the animal grows in thickness. This splitting and enlargement of one of its arms I observed in specimen No. 4 during one night in September of 1905. The worm pushed the rim of its buccal funnel nearly to the margin of the orifice, and slowly moved the ends of the tentacles over the rim of the tube. (In order to enter this narrow portion of the tube from below the edges of the buccal funnel and anterior region of the body were curved dorsalwards and considerably contracted till they became conical in form.) The animal remained in this position in the tube about five seconds, then slowly withdrew into the deeper portion. This was repeated in thirty seconds, but this time it withdrew only to the level of the sand. Here the worm suddenly expanded the first pair of setigerous segments and split the tube longitudinally at its outer side, then withdrew quickly into the deeper portion of the tube. Fifteen or twenty seconds later the worm reappeared at the level of the sand, extended the rent a little

worm in No. 4 extended its tube to the glass wall of the aquarium on May 8-9, 1906. The U-shaped tube now measured 85 millimeters between the orifices.

higher and again withdrew. This action was repeated five times in extending the rent, seven millimeters, to the end of the tube. The rent was produced by means of the expansion of the muscular, setigerous region and not by the sharp lance-shaped setæ as one might suppose. The rent occurred in a position ventral to the plastron. When the tube was split to its extremity the worm thrust one side of the anterior region through the cleft and removed the sand about it by means of its setigerous notopodia. They pressed a portion of the sand aside, but some was removed backwards into the tube and later discharged at the other end.

When the tube was split to its end the worm spread the basal portion of the rent by a slight expansion of the ventral side of its lower lip and the foremost portion of the anterior region. The worm remained in this position for fifteen or twenty seconds, then withdrew into its tube for half a minute, after which it took a position a little nearer to the orifice of the tube. The performance was repeated till the edges were reunited by a wedge-shaped insertion of parchment that widened to three millimeters just below the level of the sand. I could not determine which region of the body was most active in the secretion of the mucus, which becomes parchment-like, but I observed that it was shaped by the lower lip of the buccal funnel, and that the parchment film had advanced a little higher each time the animal applied its ventral lip to the cleft. The splitting of the tube and the closure of the rent were completed in thirty-five minutes.

The splittings occur indifferently on any portion of the circumference of the tube, but they are found chiefly on the upper side of the horizontal portion. When they are extensive it is indicated by the abundance of sand discharged at long intervals from one arm of the tube. I have found some large tubes which had strips of thin parchment two centimeters wide and as long as the horizontal portion of the tube.

The new portion of the wall is thin and membranous at first and, while it becomes thicker with age, can be observed, long after its formation, as a strip somewhat thinner than the remaining portions of the wall. Its inner surface is smooth, like the inner wall of the other portion, and its outer surface is similarly covered with sand.

The wide, horizontal portion of nearly every tube bears one or more of these strips inserted between the edges of a thicker laminated wall. This was true even in the smallest specimens, No. 2 and No. 4, which I mentioned on page 525. The diameter of their tubes was twice enlarged while they were thirty-eight and thirty-two millimeters long, respectively, and before they constructed the next linear enlargement.

The outer surface of the tubes is everywhere coated with sand, excepting about the terminal portions that protrude above the sand flats in which they are imbedded. These terminal portions have one or more annulations that give them the appearance of being formed of rings that diminish regularly in size upwards, so that the bases of the smaller rings are overlapped by the top of the ring next below. Each ring represents the successive height of the orifice, though not its diameter, for they are split from time to time as I have just shown. They are molded, like the other portions of the tube, by the ventral lip of the buccal funnel, and the length of each ring represents the height to which the lip was extended when it was formed. The rings are, at first, very thin and transparent, but they become laminated by successive additions of mucus to their inner walls. The laminæ of which they are the free ends may be separated with ease from those next below.

Summary.

The species of Chætopterus which is found at Beaufort, North Carolina, is *Chætopterus variopedatus* instead of *Chætopterus pergamentaceus* of various authors.

The cowl-like structure which is on the dorsal side of the thirteenth somite of the body is an accessory feeding-organ. It is not a "dorsal sucker" as was claimed by Laffuie.

The dorsal diverticulum of the œsophagus is provided with glandular walls. It is of the same length and diameter as the food-masses. It is possible that the food collects in it and is formed into little masses such as are found throughout the intestine.

The intestine is provided, along its dorsal side, with a ciliated groove that extends backward from the ciliated portion to the posterior

end of the animal. This is probably of use in moving the food-pellets toward the anal end of the intestine.

The annelids, so far as I was able to determine, behave in the parchment-like tubes as they do in glass tubes of about the same size and form.

The eggs and spermatozoa are discharged to the exterior through the nephridia.

The larvæ develop a mesotrochal girdle of cilia which is soon succeeded backward by a second and third ciliary girdle while the mesotrochal band becomes atrophied.

The luminosity which in the adult is associated with the secretion of mucus is early seen in the larva in the region of the ciliary girdles.

The "terminal papilla" functions as a hold-fast when the larva comes to rest on some submerged solid.

Well-fed larvæ develop three pairs of eye-spots. This was true of all the larvæ taken in the tow-net, but was met less frequently among larvæ reared from the eggs. The presence of this number of eyes led Joh. Müller to name the larvæ *"Mesotrocha sexoculata."*

The transformation is a gradual one. That portion of the larva anterior to the second and third ciliary rings becomes the anterior region of the worm. It becomes flattened horizontally and the setigerous somites appear at first as a transversely arranged series of pigment-spots on the ventral side. The post-oral lip enlarges enormously and becomes extended forward as the ventral lip of the worm. Green granules like those of the adult occur in the cells of the entoderm in the middle region of even the youngest larvæ collected in the tow-net.

The second and third ciliary girdles mark the first and second somites of the middle region. The former is carried outward by the growth of the aliform notopodia and lines the ciliary furrows of these organs. A portion of the other ciliary girdle persists within the dorsal "accessory feeding-organ." The palettes, the last three somites of the middle region of the worm, are formed by the rapid growth of the dorsal portion of the saucer-shaped somites that lie posterior to the ciliary girdles. This region is succeeded by a very

short anal somite to which the growing-zone is confined. The posterior region of the worm is formed by differentiation of this growing-zone.

The transformation is accomplished by a change of habit. The larva ceases to swim at the surface. It creeps over the bottom where it forms short tunnels the walls of which are made of sand and diatoms cemented together by mucus which is secreted by mucus-cells of the body of the larva.

The young worm remains permanently confined within one of these tunnels which it enlarges as often as may be necessary to accommodate its own rapid growth.

The tunnel is enlarged in diameter by being split lengthwise and a new wall is formed in the open space. These tubes are lengthened by the construction of an extension to one end in the form of half of a broad U. Subsequent extensions are made indifferently from one end or other of the base of the old tube. The intermediate arm, which is divided from the main portion of the tube by a transverse septum, becomes macerated and leaves the typical U-shaped tube.

The tubes are made of mucus which is secreted by the body and shaped by the anterior region of the worm. The mucus hardens to a parchment-like consistency.

The rate of growth of the worms is very rapid. Those which develop from eggs laid early in the season reach maturity before the close of the same season.

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REFERENCE LETTERS FOR FIGURES.

ac. fd. org., accessory feeding-organ.
ad. d., adhesive disc.
al. not., aliform notopodium.
an., anus.
cil. 2, second ciliary girdle.
cil. 3, third ciliary girdle.
cil. gr., ciliary groove.
cir., cirrus.
dors. div., dorsal diverticulum.
D. V., dorsal vessel.
fl., flagellum.
gl., gland-cell.
hft., holdfast.
int., intestine.
int. neur., internal neuropodium.
l. ey., lateral eye.
med. ey., median eye.
mth., mouth.
neph., nephridium.
neur., neuropodium.
neur { int. lb., internal lobe of neuropodium.
neur { ext. lb., external lobe of neuropodium.
ov., ova.
ovy., ovary.
pal., palettes.
pl., plastron.
p. o. l., post-oral lobe or lip.
pr. o. l., pre-oral lobe or lip.
set., seta.
tt., tentacle.
unc. pl., uncinal plates.
vent. nv., ventral nerve.
v. mus., ventral muscle
v. v., ventral vessel.

XII, XIII, XIV, XV, XVI, designate the names of the segments of the middle region of the worm.

PLATE I.

FIG. 1. Photograph of tubes of *Chætopterus in situ* on shoal at low tide. Reduced $^{1}/_{5}$.

FIG. 2. Photograph of parchment-like tube with lateral extension and intermediate tube. Removed from the sand. Natural size.

FIG. 3. Photograph of ♀ *Chætopterus* removed from its tube. Dorsal aspect. Shows general transparency of the integument, the forward tilting of the accessory feeding-organ of the thirteenth segment, the presence within the parapodia of the sexual segments the coiled ovaries. Reduced ⅓.

FIG. 4. Photograph of ♂ *Chætopterus* removed from its tube. Dorsal aspect. Shows the forward tilting of the accesory feeding-organ of the thirteenth segment and the milky-white color which is due to the presence of ripe spermatozoa within the parapodia of the sexual segments. Reduced ⅓.

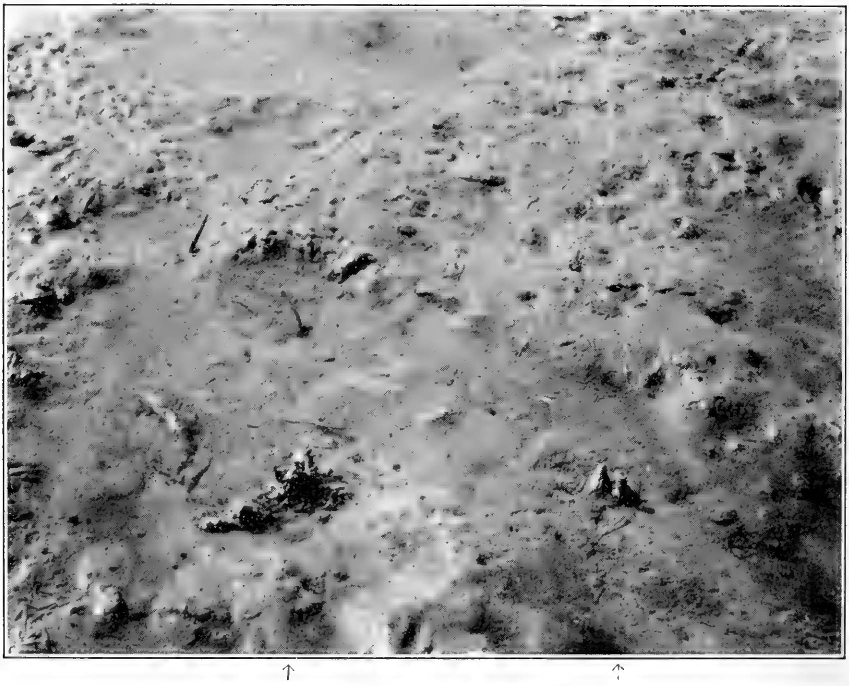

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4

Plate II.

Fig. 5. Dorsal aspect of adult ♀ *Chætopterus*. (From preserved specimen.) $\times \frac{3}{4}$.

Fig. 6. Ventral aspect of same specimen. $\times \frac{3}{4}$.

Fig. 7. Model of accessory feeding-organ of the 13th segment. $\times 3$.

Fig. 8. Posterior aspect of partially dissected sexual segment of adult *Chætopterus*. Ventral side uppermost. $\times 1\frac{1}{2}$.

Fig. 9. Ventral aspect of larva of *Chætopterus*. (Drawn from life.) Six days old. 1 mm. long. $\times 43$.

Fig. 10. Ventral aspect of larva of *Chætopterus* at beginning of the transformation. (Drawn from life.) 1.5 mm. long. $\times 31$.

Fig. 11. Ventral aspect of larva of *Chætopterus* at the time of the transformation from free-swimming to creeping stage. (Drawn from life.) 2 mm. long. $\times 23$.

Fig. 12. Same larva. Dorsal aspect. Post-oral lobe extended. $\times 23$.

Fig. 13. Same larva. Viewed from left side. Post-oral lobe depressed. $\times 23$.

Fig. 14. Posterior end of adult *Chætopterus*. Shows regeneration of the sexual segments. $\times 5$.

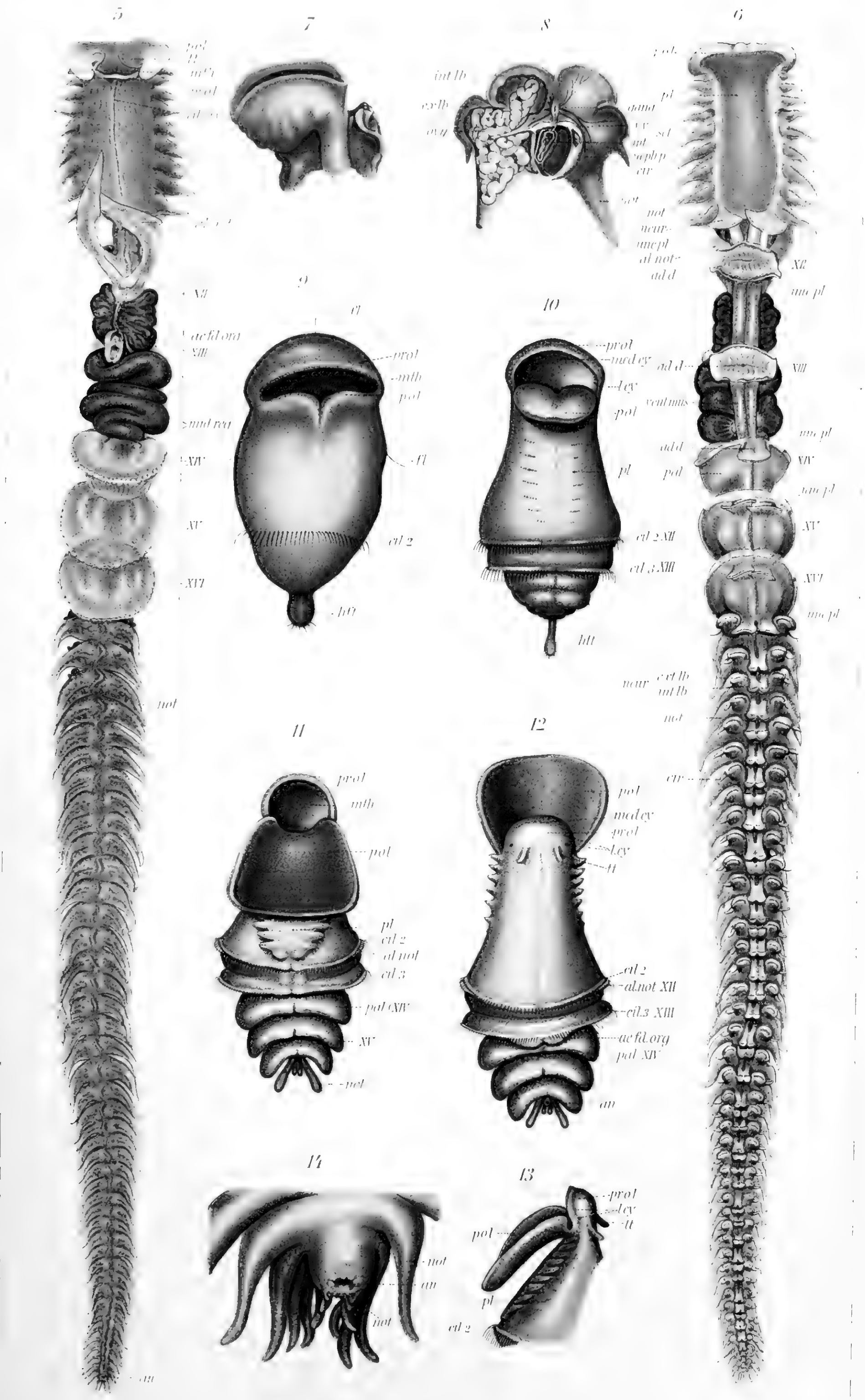

PLATE III.

FIG. 15. Transverse section through adult *Chætopterus variopedatus.* Taken through middle of anterior region. $\times$ 7.

FIG. 16. Transverse section through posterior portion of anterior region. $\times$ 7.

FIG. 17. Transverse section directly back of anterior region. Taken through narrow portion of 12th segment. $\times$ 7.

FIG. 18. Transverse section through same region. Taken nearer parapodia of 12th segment than that shown in Fig. 17. $\times$ 7.

FIG. 19. Transverse section through parapodia of sexual segment. $\times$ 7.

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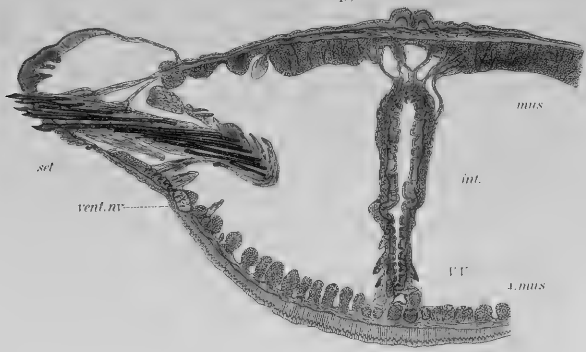
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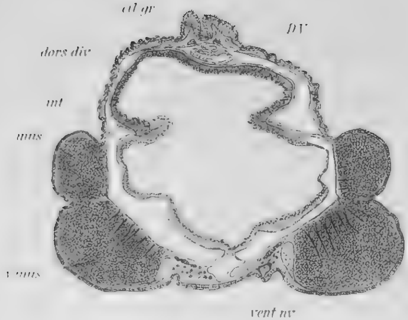
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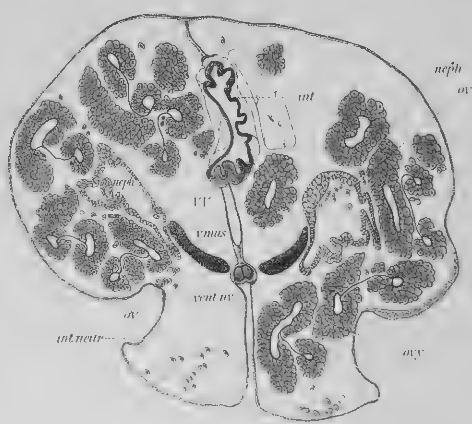
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GENERAL FEATURES OF THE EARLY DEVELOPMENT OF DESMOGNATHUS FUSCA.

WILLIAM A. HILTON.

This small salamander is very plentiful in eastern United States and in many places is without doubt the most abundant of the Urodela. It seems to be entirely nocturnal in its habits, venturing out from hiding only at night. During the day time it may be found under logs or stones on the edges of brooks, where it is neither very wet nor entirely dry. This species is seldom found in water, almost never in deep pools, although individuals may take to deeper water than usual when trying to avoid capture. In general this form may be said to be more aquatic than Plethodon cinereus and less so than Spelerpes bilineatus with which it is often associated. The egg-laying of the three species seems to be quite typical of the average habits of the three animals so far as water is concerned. The eggs of Spelerpes are laid in shallow water, those of Desmognathus in damp places near small brooks or springs, while the eggs of Plethodon are laid in a way similar to those of Desmognathus but farther from water. The larval history of the three species also illustrates the various degrees of aquatic adaptation. The larvæ of Spelerpes remain in water until they have attained a size nearly or quite equal to that of the adult before losing their external gills, that is, they retain their larval characters for a long time. The larvæ of Desmognathus live in water for only a short time; they lose their gills when very small. The young of Plethodon have a still shorter larval period, the gills are soon lost and the adult form is attained without entering the water.

The eggs of Spelerpes resemble those of frogs and toads in many ways, while the eggs of Desmognathus, Plethodon, Autodax lugubris, Necturus maculatus and a few more Urodela resemble each other in a number of features and seem to be more fish-like in their development.

The first description of any of the stages of development of Desmognathus fusca was made by Prof. H. H. Wilder, March, 1899. A few rather advanced embryos were described and figured. The embryos were found to be strikingly like those of fish, with a large yolk-sac well separated from the body of the animal. Judging from these stages there seemed to be good reason for supposing the segmentation to be like that of fish or meroblastic. In September of the same year, Ritter and Miller described the eggs and some of the stages of later development of Autodax lugubris. In this form they found the eggs to be very large, as much as 5 mm. in diameter, and the position of the embryo on the yolk-sac was strikingly like that described by Wilder for Desmognathus. No evidence was brought forward to show that the eggs were meroblastic, although such a type of segmentation was suspected. Montgomery described some rather late stages of Plethodon development and from his study it was learned that the so-called yolk-sac remained divided into distinct cells or blastomeres until a rather late stage when all the cell outlines became lost and the blastomeres were fused together.

In 1904 H. H. Wilder and the author both published short articles on the early development of Desmognathus fusca and both papers showed without a doubt that the segmentation was holoblastic and not meroblastic. Dr. Wilder's evidence was from surface views, the other was from sections as well, and in the latter it was found that although after a time the segmentation became total, at first it was not so.

The present paper is a continuation of the study of early development. Only an outline of the early stages up to embryo formation will be given. At another time I hope to be able to consider some phases of blastopore formation and later embryonic development more in detail.

The following observations on the eggs of Desmognathus fusca

were made so far as possible upon living specimens; this was possible in earlier stages, but more difficult in later development. The observations on the living eggs were confirmed and supplemented by a study of corresponding stages of preserved material with serial sections through selected embryos.

Great difficulty was experienced in the removal of the egg membranes and if these were left on, the surfaces were injured by osmosis in the alcohols. If eggs are fixed in formalin this may be avoided. Although the membranes are rather difficult to remove with instruments they may be easily dissolved by a weak solution of potassium hypochlorite. Eggs which have been preserved in formalin do not cut very well in paraffin, although they cut easily in collodion and quite well in collodion and paraffin combined. Formalin preserved eggs are good for external study, the natural colors and markings are so well retained, but they are soft and have to be handled with great care after the membranes have been removed. Perenyi's mixture as a fixing agent, in certain respects was very useful. The form was well preserved and the eggs so hard that they could be handled without danger of injury, but they were not so good for sections. The killing mixture which gave the most satisfactory results for sections in paraffin was Gilson's fluid, as the yolk remained soft after treatment with this reagent.

Practically nothing is known about the mating of Desmognathus fusca. The eggs obtained in central and southern New York were found from the last of June to the middle or last of July. The only case in which the female was found laying was early morning shortly after daylight, this same female when taken to the laboratory soon deposited two other eggs. Judging from the stage of development of a number of egg masses it seems probable that quite a number are laid at night. One bunch of eggs found late in the afternoon was unsegmented.

The eggs are about fifteen or twenty in number, usually grouped together in a single package under logs or stones in moist, or rather wet locations with the body of the female arched about them in the form of a semi-circle. The presence of the female seems to furnish sufficient moisture rather than anything else, for when she is

removed the eggs soon dry up, but if they are placed in a moist location and kept moist, they continue to develop for a time as well without the female as with. The small cavities under logs or stones where the female is half coiled about the eggs, are not far from water, usually near a small brook or spring, and in many cases are well hidden under piles of stones or deep down in the loose earth under some completely buried log or stone. In other cases the eggs and females may be found in partly deserted creek beds where the water seldom comes except at flood time. Here they may be under some of the slabs of shale which have been split off from the bed rock by frosts, but still remain in position. The first time one of these shale slabs is lifted, a half dozen females with eggs may appear.

Although eggs seem to be found most abundantly from the last of June to the middle or last of July, much depends upon the season. The most favorable time for egg-laying seems to be during rather dry weather, and the rapidity of development seems to depend largely upon humidity and temperature.

The eggs were found in a single bunch or in two bunches close together, in no case were they found wrapped about the body of the female. Almost always they were just within the semi-circle formed by the female's body. The eggs are quite large, 3.5 mm. in diameter, entirely without pigment and each one separately inclosed by jelly-like membranes which adhere with great persistency. Two membranes closely surround the egg and in preserved specimens may be separately removed, while outside of these is a much thicker, more jelly-like substance, this last quite uniformly covers the egg except at one point where it is thickened and prolonged into a sort of stalk by which the egg is attached to the similar prolongation of the outer envelope of the other eggs, so the bunch as a whole, as Wilder aptly states it, "is much like a bunch of toy balloons held in the hand of a street vender," although usually the mass of eggs is more compact than this, for in some cases the elongate portions of the outer envelope, instead of meeting at a rather common center, attach themselves to other places.

The eggs are clear white without pigment and with a very slight tinge of yellow. They are usually about 3.5 mm. in diameter and

seldom larger, although often smaller. The smaller ones develop as the larger, and as a rule pass through the different stages with greater rapidity. Freshly laid unsegmented eggs seem to be uniformly white with no polar differentiation, but when very closely examined it is found that the pole of the egg which floats up is of a pure chalky-white while the opposite pole and about two-thirds of the egg nearest it, although white, has a slight yellowish tinge. This lighter polar cap is somewhat variable and its limits are not well defined. It may be noticed in the living eggs, but it is more apparent in preserved ones. The lighter polar cap marks the position of the animal pole or region of more purely formative yolk. In microscopical section the only difference which could be observed between the two areas of the egg before segmentation was the smaller yolk granules of the animal pole.

In a study of segmentation stages, my results agree for the most part with the various cleavage stages described by Wilder in February, 1904.

In a large number of eggs collected from widely separated places during several years, I have only found a few which exhibited so regular a type of cleavage as described in Wilder's eight-cell and later stages. Even as early as the four-cell stage the irregularity in cleavage was considerable. These eggs seemed to be perfectly normal, for in many cases very irregularly segmented eggs developed into perfectly usual later embryos. Another marked difference found in the specimens which I studied, was that in the early stages the segmentation planes were much slower in making themselves evident at the lower pole than seems to be the case in those studied by Wilder. The eggs which came under my notice seemed in both of these respects more like those of Cryptobranchus as described by Bertram G. Smith in August, 1906.

Surface views alone do not seem to show all the important divisions of the egg even in early stages and little idea can be gained of how deep the cleavages have penetrated unless sections are made. A complete division of the egg into entirely separate blastomeres was not accomplished until a rather late morula stage was reached.

From $2\frac{1}{2}$ to 3 hours after the eggs are laid the first indication of

segmentation was recognized—a very slight depression near the center of the animal pole, followed by a very narrow groove which was deepest in the center and became less deep as the edge of the light part was approached. For quite a time this very minute crease in the egg seemed to remain shallow and not more extensive than the edge of the light polar disc. Figs. 1 and 2 show views of an unsegmented egg from above and from the side. Fig. 3 an early two-cell stage from above. This first line of cleavage may remain straight for some time or become slightly curved in its middle portion. It may become broader in its central portion in a short time and quite decided bead-like irregular cavities may develop along the line of cleavage, or very often one or both ends of this line near the edge of the light polar cap may develop club-like outlines due to the separation of the yolk to form deep clefts. These enlarged ends of the first segmentation plane seem to limit its extension about the egg for some time.

After about four hours, at the light pole of the egg and at right angles to the first division, the second segmentation plane makes its appearance as a very slight furrow. It is much as the first was in the beginning, but now the first cleavage line is much more prominent than the second, although perhaps the first cleavage lines do not even yet extend beyond the light pole. At first the two cleavage planes cross each other at right angles, but later there is a shifting of the partial blastomeres or of some of the granules composing them and the two first planes of cleavage have a small portion of their extent in common. Fig. 4 shows a four-cell stage in which the second division plane has just appeared and Fig. 5 shows the same egg twenty minutes later. The lighter lines in each case indicate second division planes. The first division plane in four-cell stages as a rule is much deeper than the second, but Fig. 7 is an exception. The division planes may at quite an early time present a beaded appearance due to small irregularities in the yolk along the line of their course. From the time the first cleavage plane appears, it begins to gradually but slowly extend itself about the egg, either mostly from one side or from both sides, but this extension is so slow and other divisions are so well along before any marked appearance of

segmentation could be seen at the lower pole, that from living eggs alone it would seem as though the development approached the meroblastic type, for it was seldom that any lines of segmentation were found in early stages outside the light cap of formative yolk at the animal pole. A preserved egg of a four-cell stage which has been divided into four partial blastomeres for some time is shown in Fig. 8 from above and Fig. 9 from the side turned a little; it shows the rather light second division not reaching to the edge of the white polar disc, while the first division plane is not only heavier but also much more extensive, reaching much below the white polar cap, on one side nearly two thirds of the distance from the animal to the vegetable pole of the egg and on the other side not quite so far, but at this stage the cleavages have not progressed far enough to enable one to see anything of them when the egg is viewed from the lower pole. Sections of a four-cell stage show the first division plane to be simply a rather shallow groove in the mass of yolk. The second segmentation plane is found as a somewhat narrower and shallower groove. No other indications of division could be detected in any other part of the egg. Both grooves were confined to the animal pole where the yolk granules in something less than a third of the upper pole were much finer than the rest of the egg (Fig. 49).

The conditions so far described for the four-cell stage apply to the earlier stages; later these rather regular planes of division may become irregular due to the shifting of the blastomeres or perhaps due to the movement of the yolk granules. This shifting, which is indicated in Figs. 10, 11 and 12, is often carried to an extreme so that deep wide fissures may be formed between the blastomeres as in Fig. 10. In the egg of Fig. 11, the blastomeres have shifted considerably and we have the beginning of an other division indicated by a small branch off from one of the heavier lines. In Fig. 12, it will be noticed that there are beginnings of other divisions indicated by branches from the heavier lines. An interesting point in this stage of development which perhaps may be seen better in more regular and later stages, is the absence of anything which might be called an equatorial division which is so characteristic of many amphibian eggs. Fig. 14 shows quite well how the additional blastomeres are

formed from the four-cell stage by clefts which branch off from the earlier cleavages, this represents a six-cell, and in this egg at this time the first cleavage plane has nearly encircled the egg and now for the first time there is an indication of the first segmentation plane from the vegetative pole (Fig. 15). Another egg of a similar stage of development is shown in Fig. 13. This last might be called a five-celled stage with the beginnings of other divisions which soon become more prominent. From the vegetative pole of this stage one part of the first segmentation plane is visible. What may be called an eight-cell stage seems to be rather irregularly derived from a four-cell stage by the gradual addition of furrows which in any case cannot be recognized as parts of an equatorial cleavage plane. Several blastomeres may come to be marked off in this way, their limits become well defined at the animal pole, but in all eggs examined by me they were not well defined a short distance away from it.

The original furrows of the first and second planes, although a little more pronounced than the others, can hardly be told from them, their course is so changed by a shifting of the parts; however, they are usually of greater extent and are deeper.

Quite a typical eight-cell stage is shown in Fig. 16. Fig. 17 shows the same egg from below, the first segmentation plane completely encircles the egg. Fig. 18 shows another similar eight-cell stage from the side.

Sections of an eight-cell stage show quite a large segmentation cavity under the few well defined blastomeres of the animal pole, these few central cells in the region of small yolk granules were well marked off from the rest of the egg while other divisions seem to have only penetrated a short distance into the mass of yolk. Even in the case of the first segmentation plane which nearly encircles the egg, the central mass of yolk still remained undivided (Fig. 50).

Wilder recognized a horizontal cleavage plane about the upper pole of an eight-cell stage, but in the eggs examined by me the eight-cell stage was more irregular and I could not recognize such a plane of cleavage very clearly. Now and then I obtained stages which were quite regular and approximately sixteen-cell stages such as Fig. 19, but I could trace no single regular cleavages which brought about

this result, and there were indications that the eggs I studied gradually split off blastomeres at the animal pole and came to be roughly of the sixteen-cell stage.

Fig. 20 is a later stage where a number of the cells have divided to form a rather coarse morula. In this and later stages there was no evident indication of the location of the early segmentation planes. Figs. 21, 22, and 23, show different views of the same egg in a later coarse morula stage. Fig. 23, which is from below, shows relatively few furrows at the vegetable pole. Sections of about this stage, such as Fig. 51, show the complete division of the animal pole into a number of well separated blastomeres with fine yolk granules, forming a single layer of cells over a fair sized segmentation cavity, while the vegetable pole is shown to be only partly divided into blastomeres, that is even yet the large granuled yolk mass of the vegetative pole is not completely separated into blastomeres. In this and subsequent stages it will be noticed that the most complete and most rapid division is in the region of the animal pole, where the yolk granules are smaller, but division is by no means confined to it, and the vegetative pole gradually becomes broken up into blastomeres. As the rather irregular division proceeds, the smaller cells of the animal pole become smaller and smaller, divisions of the vegetative pole continue to be deeper and more numerous and the general appearance of the egg on the surface is much like that of ordinary holoblastic eggs of frogs and salamanders; such as Figs. 24, 25, and 26. Such stages seemed to be reached in about 24 to 27 hours after laying, depending much upon conditions of temperature and moisture. In a stage such as Fig. 25, the small cells of the animal pole are very numerous, while those of the vegetative pole are quite numerous and the cleavages deep, so all parts of the egg seem to be well divided into cells. Sections of some of the less advanced fine morula stages, such as Fig. 25, show the central part of the yolk mass to be almost undivided, while at its periphery smaller cells may be seen which are those seen in surface views. In such stages the region of separate cells seems to begin at the animal pole and completely encircle a central yolk mass, the few divisions on the lower pole have not yet penetrated this central yolk. Fig. 53, of a section perpendicular

to the axis drawn through animal and vegetable pole, shows the cells on the exterior and the central undivided yolk mass. In more advanced stages of late morula the cells of the animal pole are numerous and smaller, sections show a considerable advance over conditions found in an egg like Fig. 22. The small cells of the animal pole have become very numerous, quite small and of more than a single cell layer in many places, while below these and separated by a slight segmentation cavity, the central yolk mass is shown for the first time completely divided into separate blastomeres, some of them large, it is true, but the segmentation is total. The cells above the small segmentation cavity are somewhat rounded in form and smaller than the other cells which are composed of larger yolk granules of the vegetative pole. Between and about the larger cells near the animal pole are spaces continuous with the segmentation cavity. It would seem that if many of these cells divided and became more compactly arranged the spaces between the cells as well as the rather small segmentation cavity might come to be obliterated. In later stages this is what seems to take place, but in such stages very little can be learned from surface views, the whole egg appears like an unsegmented ovum because the blastomeres are so small. In sections of a number of eggs of about this stage, the whole egg seemed to be a solid mass of rather small, large-granuled cells surrounded in large part by a rather definite more or less simple layer of small cells with small yolk granules. The extent of these small cells with the fine yolk granules was found to be quite a little greater than in the last fine morula stage recognized, and as a large part of this surface seemed to be made up of small cells, in many places of only a single layer, it seems quite probable that there is quite a migration of the small cells of the animal pole over the large yolk granule cells of the vegetative pole. There is at least a considerable rearrangement of cells to form this simple covering, but there is also a strong probability that the large central cells contribute somewhat in the formation of this small cell covering which partly surrounds the central mass.

The way in which the small cells seem to grow about the rather solid central yolk, seems to be unlike anything I have found described

for Amphibia; it seems to slightly resemble conditions which result in the development of certain stages of fish eggs.

External features of blastopore formation seem to be not unlike the similar stages of other Urodela; a minute depression marks the beginning of gastrulation, later a half ring-like groove, Fig. 28, and still later a complete ring-like blastopore comes to be formed (Fig. 29). At a still later stage only a little dimple is seen from the surface of the egg and a small mass of yolk projecting from the small opening represents a typical yolk-plug stage. No indication could be obtained from the exterior of the exact place where gastrulation began. Sections of a number of eggs during early gastrulation show various stages of ingrowth of small cells on the surface into the solid mass of yolk-cells which make up the greatest bulk of the egg. This ingrowth which may be recognized in sections of stages similar to Figs. 28 and 29, seem to start in about the central part of the area covered by small cells and seems to concern itself with the small granuled surface cells. These cells seem to multiply and push themselves down into the larger yolk-cells forming a more or less sharp cleft between them, Figs. 54 and 55. Whether this down growth is entirely confined to the multiplication of surface cells, or whether the adjoining yolk-cells contribute to some extent, it is difficult to determine from the evidence which is at hand at present. I can only say that this cleft in the cells seems to be chiefly due to the growth of the surface cells down into the mass of other larger cells which have larger yolk granules.

In a section of a later stage, such as given in Fig. 58, a small yolk-plug is shown. There has come about a rearrangement of cells in such a way that we can recognize clearly two layers of small cells on the dorsal lip of the blastopore and a thickened mass of the ventral lip. A yolk-plug of not very clearly defined, large granuled yolk cells projects between the two lips and this mass of yolk for some reason, seems different from the more definite cells which occupy the larger part of the egg. These last cells seem to be little changed from what they were in slightly earlier stages. The region occupied by the less easily defined cells, the mass of which projects from the mouth of the blastopore as yolk-plug, is well separated from

the other mass of cells and probably occupies a position in part corresponding to the future position of the archenteron. The archenteron in some early stages seems to become quite a cavity; the living egg gives no indication of the cavity, but it was easy to injure its thin roof on examining the egg, so it was often accidentally demonstrated. In preserved eggs this thin layer of cells sinks down toward the lower side of the cavity as shown in Fig. 59. In stages of this kind the blastopore remains as a small often triangular opening at what may be called the caudal end of the egg and very faint indications of a broad medullary plate make their appearance, Fig. 30. A longitudinal section of such a stage cut through the blastoporic opening shows the thin roof of two general layers, somewhat collapsed, but still with a considerable archenteron. The cells of the ventral lip of the blastopore are shown as slightly different from the general yolk cells and to some degree forming the floor of the archenteron.

In these last stages there has been no reference to a segmentation cavity because none was clearly recognized. Spaces sometimes occur between the cells of the yolk mass, possibly in some cases artifacts, but in some at least they seem to be secondary segmentation cavities developed between the blastomeres.

From the material at hand it was impossible to recognize with certainty the cavities in the egg at the time of gastrulation which Schultze recognizes in the frog. In Desmognathus there is at first the segmentation cavity or blastocele, but it seems to entirely disappear before gastrulation. Later a secondary segmentation cavity can be recognized as shown in Figs. 58 and 59, and the cavity of the archenteron is apparent. The secondary cavities which make their appearance between the yolk cells in later stages may correspond to the "Ergänzungshöhle" of O. Schultze.

Very soon after the stage described as yolk plug, or about forty hours after laying, there comes to be a very slight indication of the medullary plate from the exterior, in the large area of the egg which floats up because it is lighter. It is whiter than other parts of the egg. In the central part of this area there soon comes to be a very shallow groove and on each side of this a gentle slope of the very broad early medullary plate. This slight groove leads down to the

small nearly straight blastopore. Very soon this neural plate with its groove becomes triangular (Figs. 30 and 31). The depression of the neural groove becomes considerably deeper, definite folds are produced on each side with an open space in front; these are thick and higher in front or at the end away from the blastopore. Running down between them is the narrow median groove which was less prominent in early stages. In Fig. 31, the blastopore appears as a little triangular opening at the caudal extremity of the depression. The neural folds have not reached as far as this.

In later stages, such as in Fig. 32, the neural folds become continuous at the head end, the outline of the folds is elliptical, they change soon to become more rounded as they become higher and the outline of the cephalic end becomes more circular or truncate-elliptical, Fig. 33.

When the medullary folds begin to be prominent, the mesoderm is easily recognized as composed of two layers. It could not be traced very far toward the ventral side. In the cephalic region of the embryo figured in section, Fig. 61, the mesoderm is separated in two parts by the middle line of the notochord. Farther back in the same embryo there is unmistakable evidence that the notochord is formed directly from the dorsal portion of the archenteron (Fig. 63).

Later stages of medullary plate formation, such as Figs. 35, 36, 37, and 38, show the way in which the opposite neural folds meet, in the caudal region first and later in the head region. The embryo in these early stages seems to be quite well elevated from the yolk, the head end is rather broad from the first. Soon myotomes may be seen a little distance back from the head. The eyes bud out from the brain, gill arches are formed and the embryo comes to encircle more and more of the yolk as it grows in bulk and becomes differentiated (Figs. 40, 41, and 42). In quite a little later stage, such as Figs. 44, 45, 46, the head is well formed, the brain cavities may be seen through the transparent mass of the head, the myotomes are numerous and the head and tail ends of the embryo may be seen on the same side of the egg.

In a later stage such as shown in Figs. 47 and 48, the embryo becomes partly coiled about the egg, the eye, nasal pit, heart and

limb buds may be readily recognized from surface views. Soon after this, pigment develops and the embryo begins to make marked movements within the egg membranes. A section of this stage, Fig. 64, shows the solid mass of yolk cells much as they were seen in earlier stages, but the cell walls begin to be lost. About the whole yolk mass, a thin dark line of ectodermal tissue may be seen and under it and next to the yolk many loose mesenchymal cells.

Several interesting things are illustrated in the development of Desmognathus:

1. The segmentation is at first partial and then total.

2. The blastopore formation is much like other Amphibia from the surface, but seems to be entirely different from other forms described in the way the cells go to form the archenteron by growing down into a nearly solid mass of yolk.

3. The early separation of the embryo from the yolk mass, resembles to a marked degree the position of the embryo in certain meroblastic eggs.

I wish to thank Prof. B. F. Kingsbury and Prof. and Mrs. S. H. Gage for aid and encouragement in the preparation of this paper.

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EXPLANATION OF PLATES.

All figures in Plates I-IV are surface views of the early stages of Desmognathus fusca. All are enlarged about 10 ×.

Plate I.

Fig. 1. Egg before segmentation, view from above.

Fig. 2. Egg before segmentation from the side, the slightly lighter animal pole is up.

Fig. 3. Beginning two-cell stage from above.

Fig. 4. Four-cell stage from above

Fig. 5. Same egg as Fig. 4, twenty minutes later.

Fig. 6. Another four-cell stage from above.

Fig. 7. Another four-cell stage.

Fig. 8. Later four-cell stage from above.

Fig. 9. Same stage as Fig. 8, turned a little so as to show the extent of the first cleavage plane in the direction of the lower pole.

Fig. 10. Rather irregular four-cell stage from above.

Figs. 11 and 12. Later irregular stages of segmentation.

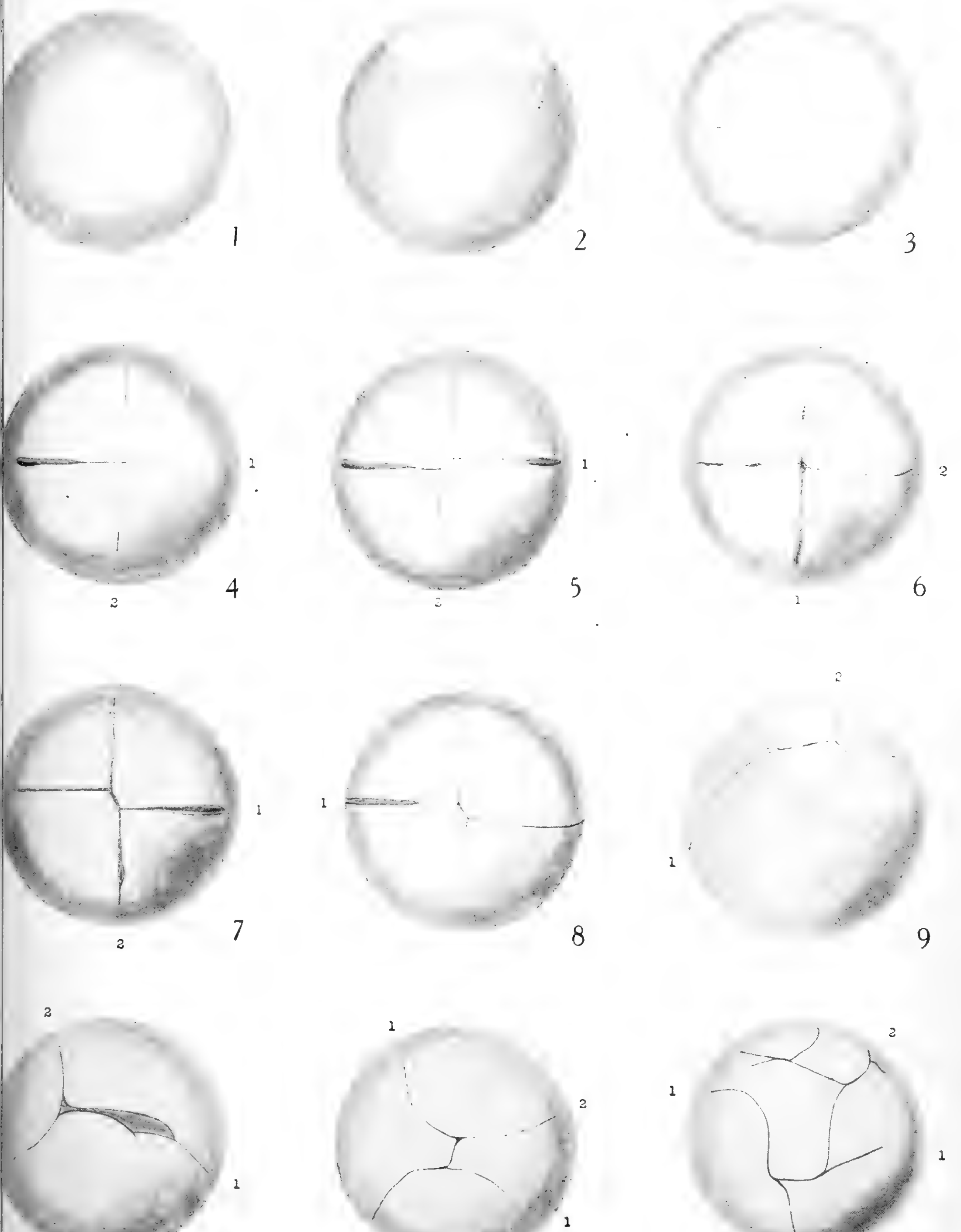
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Plate II.

Fig. 13. Rather regular five-cell stage from above.

Fig. 14. Rather regular six-cell stage from above.

Fig. 15. Same egg as shown in Fig. 14, but from below, showing the extent of the first cleavage plane.

Fig. 16. Eight-cell stage from above.

Fig. 17. Same egg as Fig. 16 but from below, showing extent of first cleavage plane.

Fig. 18. Rather regular eight-cell stage from the side.

Fig. 19. About a sixteen-cell stage from above.

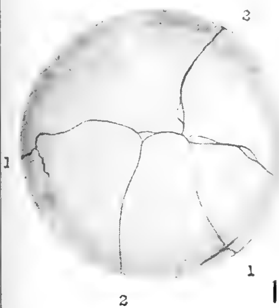
Fig. 20. Same egg as Fig. 19, later stage.

Fig. 21. Few-cell blastula from above.

Fig. 22. Same egg as Fig. 21, from the side.

Fig. 23. Same egg as Fig. 21 from below, showing some of the first segmentation planes meeting below.

Fig. 24. Many cell blastula from above.



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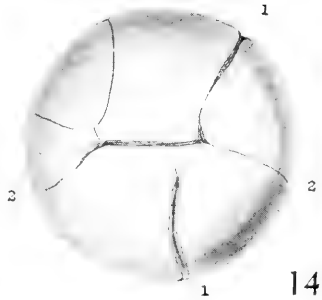

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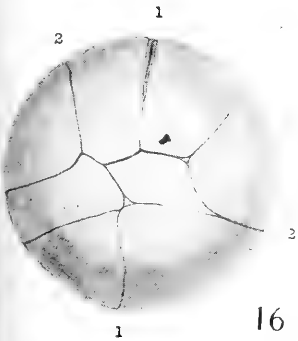

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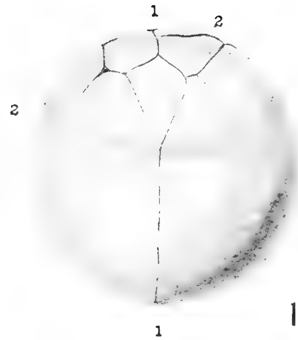

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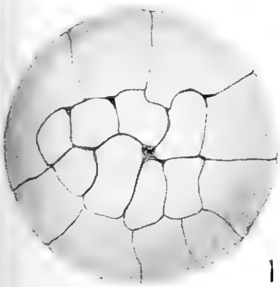

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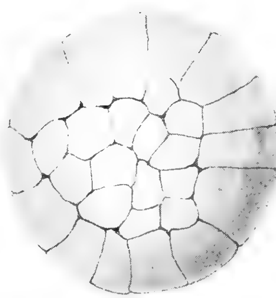

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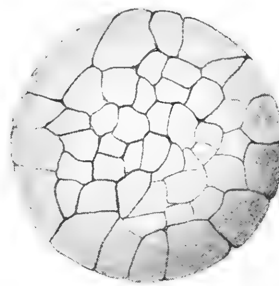

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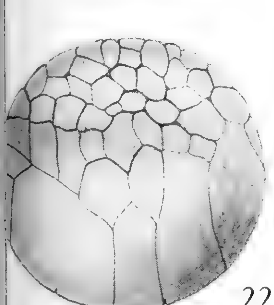

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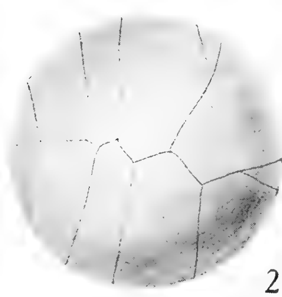

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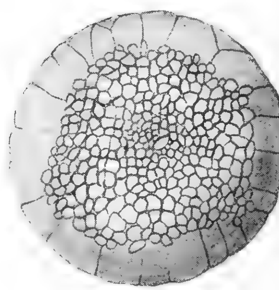

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Plate III.

Fig. 25 and 26. Two stages of many-cell blastula from the side.

Fig. 27. Same stage as Fig. 26 from below.

Fig. 28. Blastopore formation. Crescentic groove.

Fig. 29. Later blastopore stage.

Fig. 30. Very early medullary plate, blastopore remains as a small slit.

Fig. 31. Early medullary groove, medullary folds forming.

Fig. 32. Medullary folds forming.

Fig. 33. Later stage than Fig. 32.

Fig. 34. Caudal end of stage shown in Fig. 33. Closure of neural folds at the caudal end.

Figs. 35 and 36. Later stages of closure of neural folds.

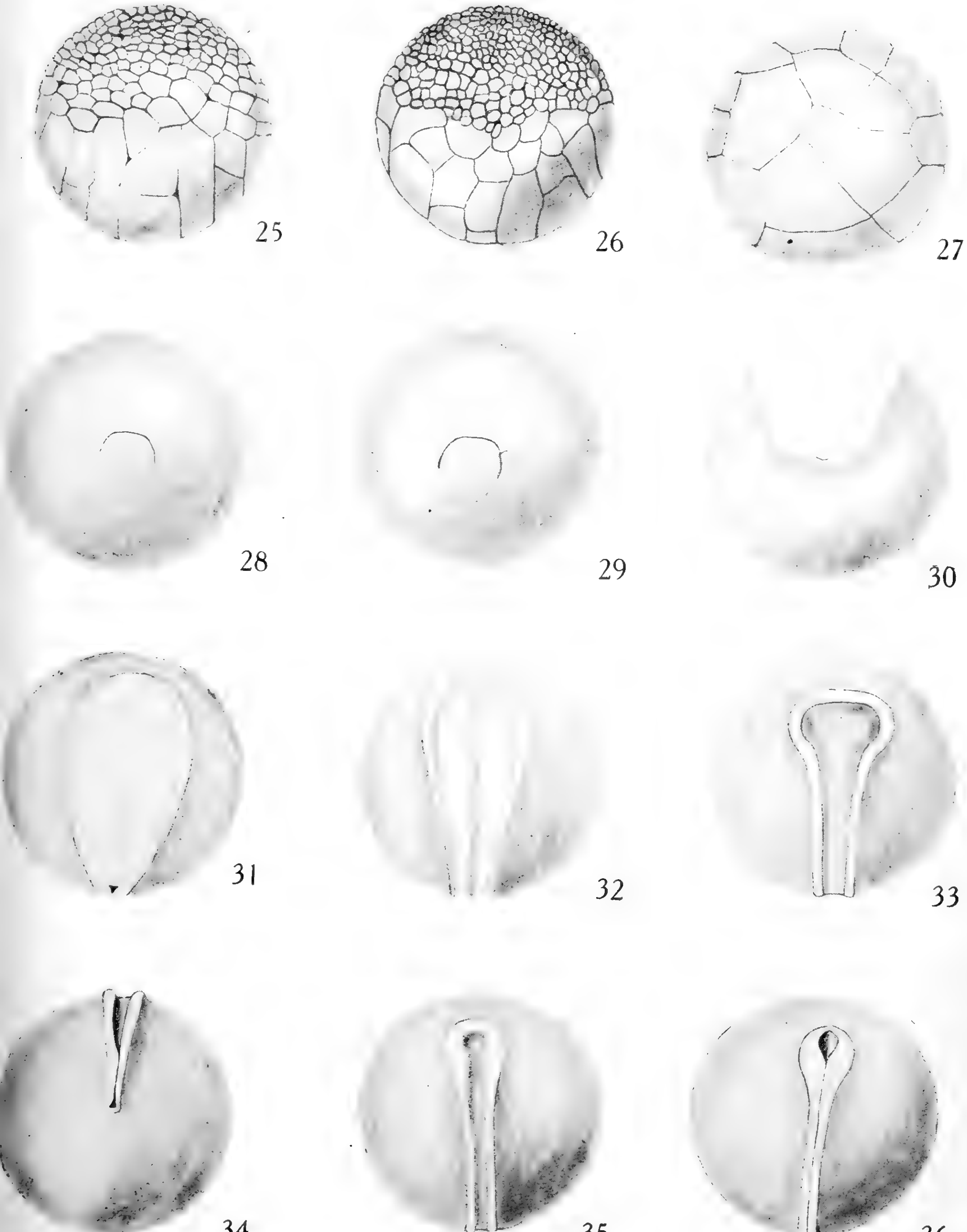
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Plate IV.

Figs. 37 and 38. Later stages of closure of medullary folds.

Fig. 39. Caudal end of stage shown in Fig. 38.

Fig. 40. Beginning of head, formation of myotomes.

Fig. 41. Later stage than Fig. 40. First indication of gill arches.

Fig. 42. Same stage as in Fig. 41. Caudal end of the embryo.

Fig. 43. Later stage, eyes well formed, also gill arches.

Fig. 44. Head of a later stage.

Fig. 45. Same stage as Fig. 44, turned so that both head and tail ends are seen.

Fig. 46. Same stage as Fig. 44, back view showing myotomes.

Figs. 47 and 48. Later embryonic stage, two views. In this stage, the eyes, nasal pit, gill clefts, heart and limb buds, may be seen.

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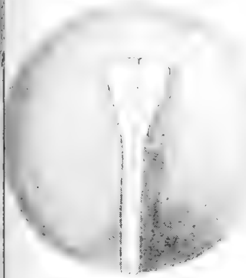

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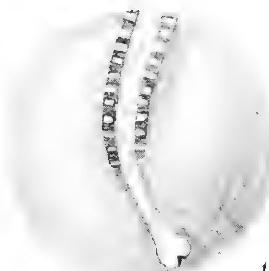

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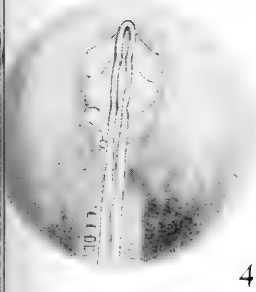

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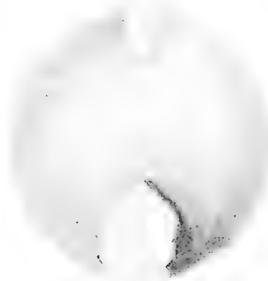

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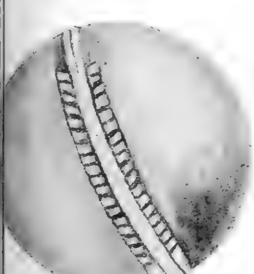

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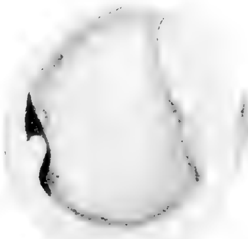

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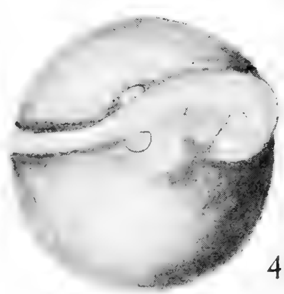

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Plate V.

Fig. 49. Section of a four-cell stage, showing small yolk granules at the animal pole and the relative extent of the first and second cleavage planes. × 13.

Fig. 50. Section through an eight-cell stage showing partly segmented lower mass of yolk and rather large segmentation cavity. × 13.

Fig. 51. Section of a few-cell blastula stage such as shown in surface view in Figs. 21, 22 and 23, Pl. II. In this stage the cells with small granules of yolk have divided a number of times, but the larger mass of the egg is only partly divided by external cleavages. × 13.

Fig. 52. Section of a many-cell blastula, such as shown in Figs. 26 and 27, Pl. III. × 13.

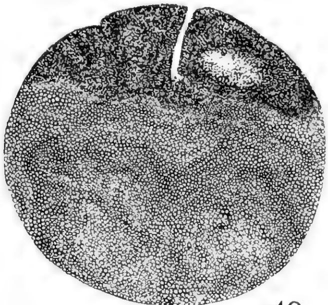
Fig. 53. Section through a small portion of the vegetative pole of an egg in a slightly earlier stage than that shown in Fig. 51, or about the stage shown in Fig. 25, Pl. III. In this stage the surface of the egg seems to indicate a total division of all parts into blastomeres, but this section shows that the central mass of yolk has not yet become segmented. × 47.

Fig. 54. Section of a later stage than Fig. 52, no segmentation cavity, a large portion of the central yolk cells surrounded by smaller cells, and in one place there is a considerable down-growth of these smaller cells into the larger yolk cells. This is a section of the blastopore stage shown in Fig. 28, Pl. III. × 13.

Fig. 55. Enlarged portion of the invagination shown in Fig. 28, Pl. III. × 55.

Fig. 56. Bunch of eggs photographed under water.

Fig. 57. Photograph of later stages of embryos with yolk sac attached.



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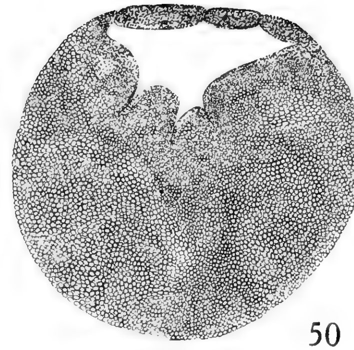

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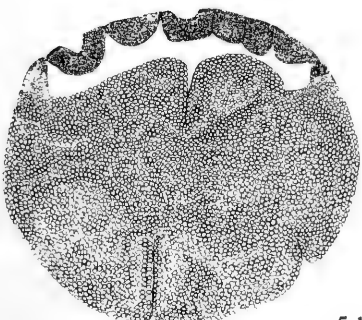

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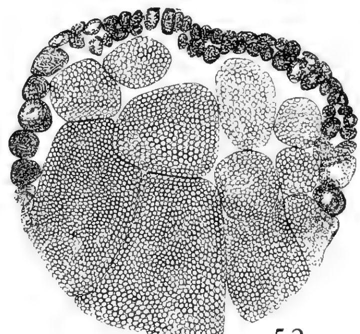

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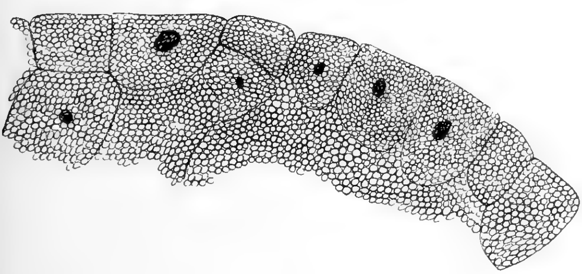

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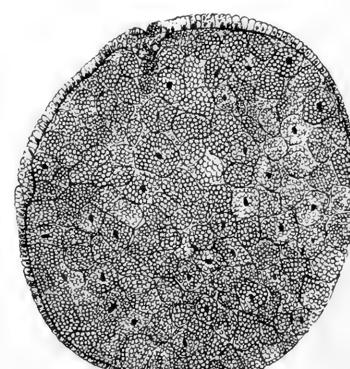

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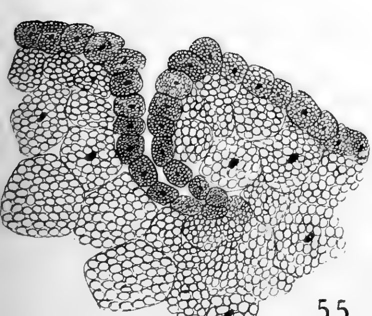

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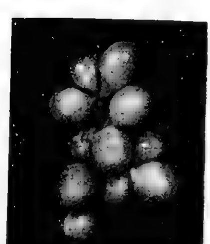

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Plate VI.

Fig. 58. Section of yolk-plug stage. Dorsal lip above, longitudinal section. $\times$ 13.

Fig. 59. Late blastopore, early medullary plate stage, longitudinal section through the blastopore. Shows cavity of the archenteron surrounded by small cells with small yolk granules. $\times$ 13.

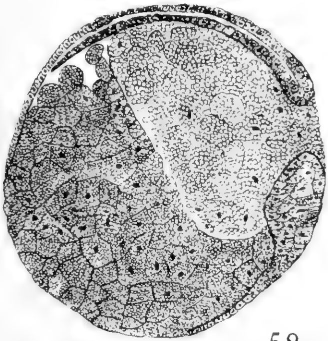
Fig. 60. Section through the blastopore of stage shown in Fig. 59, showing the dorsal lip above of two parts, also ventral lip of small cells. $\times$ 47.

Fig. 61. Section through later medullary plate stage such as shown in Fig. 33, Pl. III. In this section the beginning central nervous system, the notochord, mesoderm and small slit of the archenteron may be seen. Near the center of the yolk cells is what seems to be a secondary segmentation cavity. $\times$ 13.

Fig. 62. Dorsal portion of Fig. 61, enlarged. Showing nervous system, notochord, two sheets of mesoderm on each side and cavity of archenteron. $\times$ 47.

Fig. 63. Caudal end of medullary plate stage showing the formation of notochord from archenteron. $\times$ 47.

Fig. 64. Section of early embryo of the stage shown in Fig. 48, Pl. IV. $\times$ 13.



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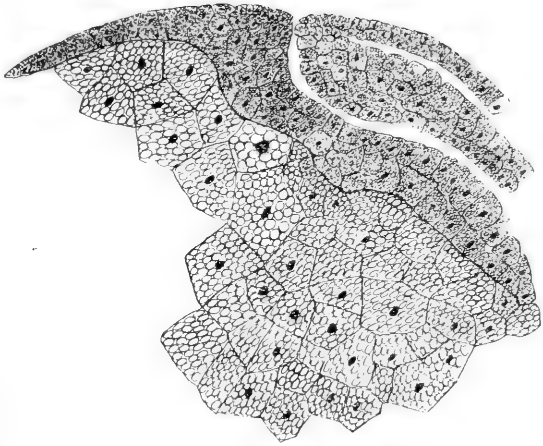

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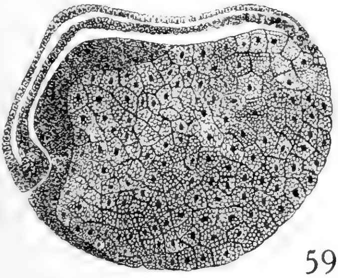

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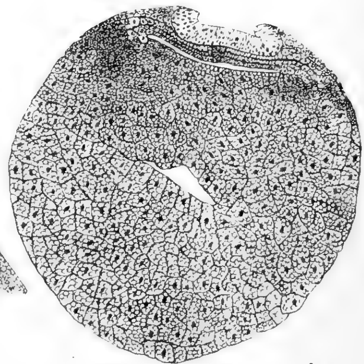

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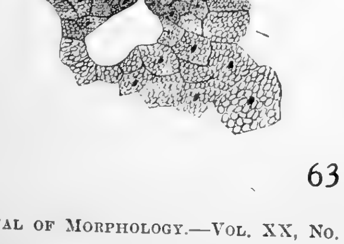

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THE COLUMELLA AURIS IN AMPHIBIA.

Second Contribution.

B. F. KINGSBURY AND H. D. REED.

Table of Contents.

In 1908 the authors published a partial report[1] upon the morphological relations of the so-called Sound-transmitting Apparatus in representative Urodeles with a view to establishing the type or types

[1]Anatomical Record, Vol. II, No. 3, June, 1908, pp. 81-91.

and their modifications. In this study the following possible general aspects were kept in mind: (1) The primary (primitive) and secondary character of the connections and relations of the structures occupying the *fenestra vestibuli* more generally designated as constituting the Columella auris, (2) comparison with the forms below (fishes) the amphibian group (the hyomandibular homology), (3) homology with the reptilian Columella and mammalian Ossicula auditus, (4) the comparison and homology in Urodela and Anura, (5) the relationships of the different urodele families, (6) the function and functional importance of these structures in the tailed amphibia, (7) the variation in the arrangement of the parts of the apparatus and its significance.

It is not necessary to repeat here the reasons that led us to believe an extensive re-examination of the comparative morphology of the structures fitting into the *fenestra vestibuli* in urodeles to be necessary, as they were briefly set forth in the first paper and previously by others (Gaupp, '98; Drüner, '03). In the examination of the different forms, there have been kept in mind the following morphological relationships:

1. Relation (connection by fusion, articulation or ligament) of the Columella (stilus columellæ) to
   (*a*) the os. squamosum (paraquadratum, Gaupp),
   (*b*) os. quadratum or palatoquadratum,
   (*c*) hyoid (ceratohyale);
2. Relation to the carotid artery (*Arteria carotis interna*) and jugular vein (*Vena petroso-lateralis*);
3. Relation to the otic capsule (lips of the *fenestra vestibuli*);
4. Relation to facial nerve (*ramus hyomandibularis* VII);
5. The ligaments coming into relation with the parts involved,—
   (*a*) *Ligamentum squamoso-columellare*,
   (*b*) *Ligamentum hyo-suspensoriale*,
   (*c*) *Ligamentum hyo-mandibulare*,
   (*d*) *Ligamentum hyo-columellare*;
6. *Musculus opercularis.*

Since early in the investigation it became apparent that there were two morphologically distinct elements appearing in the different forms, comparable, at least, if not homologous (*vide subseq.*) as we believe with the plectrum (columella) and operculum in the frog, it

became necessary to keep in mind the development of each element in its relation to the ear capsule. In the present paper a *detailed* presentation of the developmental changes, even as far as they have been worked out, is not included.

In addition to the forms briefly described in the first publication made by us, there have been examined by means of serial sections through the head, 14 species representing 1 family and 12 genera. The total number comprises 8 families, 21 genera, and 23 species, in the case of all save 9 of which the structure and morphology in both larvæ and adults were investigated. Bearing in mind that the total number of the families and genera of tailed amphibia is 8 and 45 respectively, it may be seen that a comprehensive and at the same time detailed view has been gained of the relations of the urodele "sound-transmitting" apparatus.

Without entering upon a full discussion of the bearing of our investigation, which is reserved for the last portion of this paper, the more important results may be stated briefly at this point.

1. In the tailed Amphibia, there may be developed two separate fenestral elements fitting into the *fenestra vestibuli.* These have been termed by us *Columella* and *Operculum* respectively.

2. The *Columella* typically possesses a *Stilus* which is connected primarily with the ventral (or ventro-caudal) edge of the squamosum. The *Stilus columellæ* may secondarily become joined to the palatoquadrate.

3. In development its proton appears outside the otic capsule as a group of cells continuous with the cells between the otic process of the palatoquadrate, squamosum and ear capsule.

4. The facial nerve in the forms examined is entirely below (and in front of) the columella save in Necturus, Proteus and Typhlomolge, in which the *Ramus jugularis VII* passes above the squamoso-columellar connection.

5. The *Operculum* is developed out of the otic capsule. Its position relative to the columella is caudal and medial. It possesses no skeletal connections but gives attachment to the *M. opercularis.*

6. In one group of forms, the columella only is present. In the Ambystomidæ the columella is present during the larval period but

becomes fused with the ear capsule at transformation when an operculum is formed. A further reduction and fusion of the columella is encountered in Salamandra, Triton and Diemictylus (Salamandridæ and Pleurodelidæ, Cope). In the Plethodontidæ and Desmognathidæ the fenestral plate of the columella embodies a representative of the operculum which is not separately developed.

7. The hyomandibular homology of the Columella is strengthened.

How widely the results of a detailed examination of a large number of forms depart from the interpretations at present accepted based upon the available information as to the morphology in a more limited number of salamanders, may be seen from a comparison of the above with the statements in two of the standard works: The Comparative Anatomy of Vertebrates, by Dr. Robert Wiedersheim, and the section upon the development of the skull, by Dr. Ernst Gaupp, in Hertwig's Handbuch der Entwickelungsgeschichte der Wirbeltiere.

The former ('06) describes[2] the *fenestra vestibuli* as filled by a cartilaginous plate, the so-called *Stapedial plate* or *Operculum* which is joined to the Palatoquadrate and Paraquadrate (squamosum) by ligaments, cartilage or bone. This bridge between the stapedial plate and the quadrate (or paraquadrate) is called *Columella* and together with the *Operculum*, in a phylogenetic sense, corresponds with the proximal segment of the hyoid arch (hyomandibulare or possibly symplecticum). Ontogenetically such a relation does not occur, both operculum and columella arising by differentiation in the territory of the otic capsule.

Professor Gaupp ('05) in Hertwig's Handbuch (pp. 696, 697, 605) designates the entire "stapedial" element as *Columella auris,*

[2]"Sie [Fenestra ovalis] wird von einem durch Bandmassen oder auch durch Knorpel oder Knochen an das Quadratum und Paraquadratum befestigten Knorpeldeckel, der sog, *Stapesplatte* (St) oder dem *Operculum*, verschlossen und soll uns bei der Anatomie des Gehör-Organs wieder beschäftigen. Jene zwischen Stapesplatte und Quadratum resp. Paraquadratum sich erstreckende Brücke heisst *Columella* und entspricht zusamt dem Operculum in phylogenetischer Beziehung dem oberen Abschnitt des *Hyoidbogens*. Ontogenetisch ist von diesen Beziehungen nichts mehr nachzuweisen, sondern es handelt sich sowohl für die Columella als für das Operculum hinsichtlich ihrer Entstehung um Differenzierungsprocesse im Bereich der Labyrinthkapsel."

consisting of an *Operculum* which may or may not bear a process, the *Stilus columellæ*. The process when present is usually connected by means of a ligament (Ligamentum suspensorio-columellare) with the Paraquadratum, Palatoquadratum or with both. The facial nerve in most forms passes below the suspensorio-columellar connection. It is stated, however, that there are two such connections, one above and one below the facial nerve. The operculum with a stilus is regarded as the more primitive condition and as such is probably to be homologized with the Hyomandibulare. In the Anura the Columella auris consists of two elements, *Operculum* and *Plectrum*, the latter (*Pars interna plectri*) probably to be interpreted as a stilus which has become secondarily dissociated from the operculum.

Nomenclature. The question of nomenclature is a perplexing one. Gaupp has termed the entire fenestral structure, Columella auris, consisting in Anura of two elements, Operculum and Plectrum, in Urodela of Operculum and (typically) its process, Stilus Columellæ. The direct application of the terms used by Gaupp, though desirable, becomes difficult in the light of the existence of a stilus-bearing fenestral plate and a non-stilus-bearing fenestral plate in the same form and at the same time. While there are objections to both the terms *Columella* and *Operculum*, it seemed better to avoid the introduction of new terms and to employ the term *Operculum* for the *stilus-free* structure and to restrict the name *Columella* to the *stilus-bearing* element. This use of terms seems to necessitate less departure from the earlier use of the names. The operculum of Anura is directly comparable with that which is termed by us operculum in the Urodela. This in Siredon was termed *Operculum cartilageneum* by Windischmann in 1831 and subsequently has often been designated Operculum in other salamanders. Columella was, of course, applied to the anuran structure now named *Plectrum* by Gaupp, and to the stilus in Urodeles, or to the entire element (Hasse, '73) called columella by us.

As an indifferent name for bony or cartilaginous plate fitting into the *fenestra vestibuli* we employ the term *fenestral plate.* A backward extension of the *Cavum perilymphaticum* beneath the operculum we designate *Recessus perilymphaticus.* For the bulging por-

tion of the ear capsule associated with the outpocketing of the perilymphatic space we employ the name *Prominentia perilymphatica.* Without going into a discussion of homologies, we designate as Os squamosum the Os paraquadratum of Gaupp, but in the use of other names we adhere to the terms used by him. The term *proton* is used as the equivalent of the German word *Anlage.*

We depart from the usages of the preliminary paper[3] in substituting *Vena petroso-lateralis* for *Vena jugularis interna* of which it is a direct continuation, employing the name used by Drüner, likewise using his term, *Musculus cephalo-dorso-mandibularis* for *Muscularis depressor mandibuli.* *Palatoquadratum* is substituted for *Quadratum.*

Acknowledgments. We have received material for study and helpful criticism from many sources and wish especially to express our indebtedness to Professors Wilder and Gage and Dr. W. A. Hilton of Cornell University, Professor Robert Wiedersheim of the University of Freiburg and Mr. B. G. Smith of Syracuse University. Aside from personal obligations we wish to express our appreciation of the works of Wiedersheim and Gaupp in this field of research.

Ambystomidae.

Ambystoma[4] *punctatum.* Two reasons determined the choice of this form as one of the species in which to work out quite thoroughly the development of the ear region: (a) the ease with which a large number of stages, larval, transforming and adult, could be procured; (b) its systematic rank as a typical urodele. In all, some forty series were examined and four models prepared. In this way was obtained a complete history of the transformations through which

[3]*Errata.* At this point we desire to correct errors that were allowed to creep into manuscript and proof in the earlier paper:

(1) p. 83, line 2, Hemidactylum should read Hemidactylium.
(2) p. 83, line 11, 4-6 mm. should read 12-14 mm.
(3) p. 83, line 17, 1888 should read 1879.
(4) p. 86, line 2, caudal should read dorso-caudal.
(5) p. 87, line 4, Hemidactylum should read Hemidactylium.
(6) p. 87, Fig. 4, D.e. should read D.p.—Ductus perilymphaticus.

[4]The same as Ambystoma according to older usage.

the structures under consideration pass. The results of the study in this species were given in concise form in our first or preliminary paper. The evidence upon which the conclusions were based was not submitted at that time, however, and may be given now, together with such further details as are important in this connection.

The first definite trace of the columella is to be found in embryos of 11-13 mm. in length, a short time before hatching. At this period while the cupola of the otic capsule is chondrified, the basal plate is still in the pre-cartilage (chondroblastema) stage so that the ventral boundary of the primary fenestra is not yet sharply defined, and the difficulty of delimiting its ventro-medial side is further increased by the fact that the cells of the future floor as well as those occupying the site of the future membrane are equally rich in yolk granules, in which respect they differ from the cells of the surrounding mesenchyma. The squamosum is just appearing as a minute scale of bone above and behind the otic process of the palatoquadrate.

Below and beneath the squamosum, filling in the space between otic capsule, squamosum and otic process of the palatoquadrate, and extending down upon the outer side of the vena petroso-lateralis to the yolk-bearing cells occupying the site of the future membrane, is a dense tissue, with numerous cells and homogeneous matrix, which embodies the proton of the columella, stilus and squamoso-columellar ligament. The derivation of these cells was not definitely determined. Younger embryos lend some support to the view that they migrate down around the vein. The different appearance of the cells of the otic capsule adjoining the lower end of this cell-group argues against their origin from this portion of the otic capsule (membrane or floor).

In a 13-14 mm. specimen the demarcation of the columellar tissue is sharper and the portion against the fenestral membrane denser. The condition at this stage is reproduced in a photograph, Fig. 39, Pl. IV, *col.* At about this time, chondrification begins and in the larva 14-15 mm. in length there is a small cone of cartilage, whose base is against the membrane and whose apex projects out into the dense tissue still connecting with the cell-group between squa-

mosum, otic capsule and otic process of the palatoquadrate. The stilus appears to chondrify out into this tissue, the unchondrified portion of which becomes the Lig. squamoso-columellare.

The development as given above agrees completely with the concise description of Killian ('90) for the development in Siredon. He terms the columella *Operculum* and had no knowledge of the interesting changes occurring at transformation.

During the period of larval growth the portion of the columella fitting into the membrane increases greatly in size, becoming an elongated plate of cartilage which for purposes of convenient reference may be designated *Fenestral Plate*. If at first it is outside the fenestral tissue, it becomes in the process of growth a part of that structure. Whether the enlargement of the plate takes place through marginal growth with the incorporation of fenestral tissue, or through interstitial growth with a simple displacement of the bordering membrane, has not yet been determined. In late larval life the columella apparently becomes joined to the dorso-cephalic margin of the fenestra by delicate cartilage and subsequently more closely connected with the ventro-cephalic edge. Ossification appears late. Two plates of bone are formed, one upon the inner surface of the columella, the other upon its outer surface also extends out upon the stilus. This method of ossification of the columella is quite characteristic for other urodeles as well. Fig. 33 (Pl. III) illustrates the development attained by a 35 mm. larva.

The condition shortly before transformation is shown in Fig. 22 (Plate I), from the model of the ear region in an individual about 45 mm. long. The fenestra vestibuli (*F. v.*) whose complete outline cannot of course be seen from the figure, is an elongated oval whose dorsal border is formed by the crest of cartilage where the lamina horizontalis of the lateral semicircular canal passes into the lateral wall. For purposes of reference we shall refer to this as the Crista semicircularis (Cr. s.). At the cephalic end of the fenestra the "lips" join to form an elongated prominence with which the processus basalis palatoquadrati articulates. Its interior is occupied by a cephalic extension of the cavum perilymphaticum. The caudal end of the fenestra attains nearly the end of the caudal

cupola of the otic capsule. It may be noted that the fenestra vestibuli is in the lateral wall of the ear capsule, becoming more ventral in its cephalic portion.

The fenestral plate occupies the larger portion of the window, being most widely separated from the margin of the fenestra at its caudal end. In the model the relations of the arteria carotis interna and vena petroso-lateralis are shown crossing and partially covering the fenestral plate. In Fig. 23 (Plate I), however, in a somewhat later stage, the complete outline of the fenestral plate can be seen. At the cephalic end, on both ventral and dorsal margins, new cartilage, not shown in the model, is just beginning to join the columella to the margin of the fenestra vestibuli. The stilus and ligament connect the fenestral plate with the ventral edge of the squamosum. The palatoquadrate cartilage does not become connected with the ligament. A blunt process of the quadrate underlying the squamosum is not brought into relation with the ligament at this stage.

At transformation the fenestral plate becomes joined to the lips of the opening on all sides save the caudal and the caudal portion of the ventral side. There is, however, a portion of the primary fenestra behind the stilus where even in the fully grown salamander the wall of the otic capsule is very thin,—membranous or filled in with a thin lamina of cartilage (See Fig. 24, Pl. I, "F"). In Fig. 23 the outlines of the old fenestral plate still shows. The delicate new cartilage just forming was not modeled. In Fig. 24, the fusion of the plate with the edge of the fenestra is nearly complete. The outline of the old columella and the newly formed cartilage can be distinguished.

In the process of transformation, due to the shifting of the suspensorium, the attachment of the stilus becomes largely transferred from the squamosum to the palatoquadrate cartilage (Pl. I, Fig. 24).

The operculum present in the transformed salamander (Pl. I, Fig. 24, *Op.;* Pl. II, Fig. 25) occupies the caudal portion of the new fenestra (definitiva) whose dorso-cephalic margin includes the cephalic end of the operculum; the caudal end projects freely and to it is attached the M. opercularis whose tendon fits into and fills a depression upon its external surface (Pl. IV, Fig. 35).

The operculum is formed out of the wall of the ear capsule upon the medial side of the caudal portion of the fenestra. Fig. 23 shows the operculum in process of formation. Along the line marking the medial border of the new fenestra the cartilage breaks down and is absorbed, cutting out in this way a large plate from the ventral wall near the caudal end of the ear capsule. In this histolysis, while the end result is a backward and medial extension of the fenestral opening, it is not accomplished by an actual extension back of the fenestra, but the cartilage is absorbed all along the line of separation, several irregular clefts appearing first which afterwards become confluent with each other and with the fenestra. At its caudal end the separation of the operculum comes later and at this point a new formation of cartilage occurs extending the operculum in that direc-

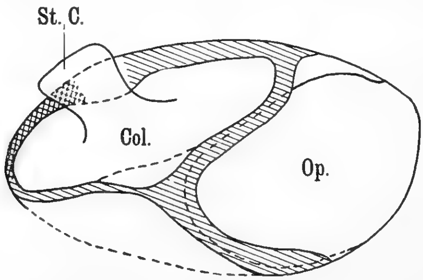


Fig. 1. Schema illustrating the fusion of the columella with the ear capsule.

tion outside the ear capsule. New cartilage is, however, formed at the edge of the fenestra as well and in the operculum itself which attains massive dimensions in the fully grown Ambystoma (Pl. IV, Fig. 35).

The inclusion of the cephalic end of the operculum by the lips of the fenestra (Pl. I, Fig. 24) is accomplished by a deposit of new cartilage which cements in solidly the fenestral plate and extends the fenestral lip backward outside the operculum. The dorso-cephalic portion of the margin is thus formed by the ventral and caudal edges of the larval columella extended by new cartilage formation, while the ventro-cephalic margin is new cartilage formed upon the ventral lip of the primary fenestra which extends to meet that deposited on the fenestral plate. The diagram, Fig. 1, will

illustrate the transformation and the filling in of the primary fenestra and the incorporation of the fenestral plate. The columella, stilus columellæ, and operculum are indicated by *Col., St. C.,* and *Op.* respectively; the new cartilage is line-shaded, while the region of earliest fusion is cross-hatched. The outline of the included portion of the operculum is marked by a broken line.

In the larva approaching transformation (Fig. 22, Pl. I), the operculum is not well outlined, although a slight groove causes a prominence of the wall which later becomes the operculum. As shown in Fig. 31 (Pl. III), in the sections of the ear-capsule of mature larvæ, the region from which the operculum will form, may be recognized. Compare Figs. 31 and 32, in the latter of which the histolysis of the thin cartilage on the medial side of the future operculum is severing its continuity with the remainder of the floor of the otic capsule. The operculum thus arises from a portion of the floor of the ear capsule while the secondary fenestra with the plate of the columella is in the lateral wall. (Cf. Figs. 32 and 33.)

The interior of the ear capsule, opposite the region to become the operculum, is occupied by the cavum perilymphaticum (Figs. 31 and 32, Pl. III). In the transformed animal this portion of the ear capsule becomes somewhat prominent and may be termed the Perilymphatic Prominence (*Prominentia perilymphatica*) (Figs. 30 and 35). The outward and backward extension of the perilymphatic space on the inner side of the operculum, as stated above, is termed *Recessus perilymphaticus.*

The muscle attaching to the operculum to which Gaupp ('93) gave the name of *M. opercularis*[5] extends caudad and at its caudal end is attached to the outer side of the *Suprascapula.* In the larval Ambystoma it is not present. Early in the transformation myoblasts upon the outer edge of the ventral spinal musculature (M.

[5]This muscle in the frog was regarded as a portion of the "Occipito-subscapularis" by Dugès ('35), the Levator anguli scapulæ of Ecker. Cope ('88 a and b) has termed it M. stapedius, Iwanzoff, M. protrahens scapulæ. Gaupp subsequently ('96) designated it the "Pars opercularis of the M. Levator scapulæ superior." In Urodela it seems to deserve recognition as a distinct muscle, and its development apparently differs somewhat in the two groups.

intertransversarius capitis inferior of the frog, Gaupp, '96) develop into a fascicle of small fibers which becomes separated off from the above mentioned muscle and gains the attachments to the developing operculum and the suprascapula. Its opercular attachment is shown in the models pictured in Figs. 23 and 24 (Pl. I) and also in Fig. 37 (Pl. IV) in which both attachments are shown.

In accord with the statement of Hasse ('73), the facial nerve is entirely below (in front of) the columellar-squamosal connection, the descriptions of other authors who have given its relations being therefore incorrect. In Fig. 22, the three branches of the hyomandibular division, ramus mandibularis internus, ramus mandibularis externus, and ramus jugularis, are shown. Joining the R. jugularis is the R. communicans IX (not shown) which pursues a course dorsad to the carotid artery to join the R. jugularis where it passes under the ligament.

In Ambystoma, therefore, there are two fenestral structures which succeed one another at transformation. The first which appears in the larva, is the columella, whose stilus connects with the squamosum and whose fenestral plate becomes associated with the otic capsule at its cephalic end. At transformation the columella becomes fused with the ear capsule and a second fenestral plate, the Operculum, is cut out. This second structure is caudal and medial to the first, possesses no process (stilus), is not connected with the squamosum or palatoquadrate, but is joined by means of the M. opercularis to the pectoral girdle.

The examination by earlier workers of the otic region in Ambystoma or Siredon,—which is to be regarded as a permanent larva of an Ambystoma,—has not lacked suggestion of the interesting condition found in this form. In Siredon, in which the condition has usually been examined, Hasse ('73), Friedenreich and Gegenbaur ('49), as well as Wiedersheim ('77), Parker ('77), and Retzius ('81), described a well ossified "operculum" of conical form, the base of the cone fitting into the fenestra, the apex of the cone prolonged into a bony process attached to the palatoquadrate cartilage by ligament (Gaupp, '99, p. 1040). The plate that fits into the window possesses, according to Retzius, a cartilaginous border and

the tip of the stilus is likewise of cartilage. This description of the condition in Siredon corresponds fairly well with the relations of the columella in Ambystoma before transformation. The names applied to the structure varied: Operculum (Wiedersheim); Columella (Hasse); Stapes (Parker). Windischmann ('31), whose paper we have not been able to examine, described in Siredon a stilus whose (fenestral) plate he termed *Patina,* while behind the *Patina* there was an *Operculum cartilagineum* joined with it only by connective tissue. This description of Windischmann's was discredited by later workers. It is probable, however, in view of the possibility that many, perhaps most, of the so-called Siredons were larval Ambystomas and not super-larvæ (Axolotl) that Windischmann correctly described the condition in a transforming individual. More recently Iwanzoff ('94) describes the condition in Siredon as follows, apparently erring in ascribing the connection of the columella with the squamosum to the operculum:

"Bei Siredon befestigt sich der Musculus protrahens scapulæ mit seinem proximalen Ende am rundlichen Knorpel auf der hinteren Wand des Schädels. Parker und Wiedersheim halten diesen Knorpel einfach für einen Teil des Knorpelschädels, um so mehr, da er schwach von dem letzteren abgegrenzt ist. Aus dem nachgewiesenen Grunde nehme ich ihn für den Stapes, um so mehr, als er demselben auch nach seiner Lage entspricht. Desshalb ist der Teil, welchen die genannten Autoren als Stapes annehmen, nämlich die teils knöcherne, teils knorpelige und bindegewebige Bildung, die vom Stapes zum Quadratknorpel und Squamosum, und dieselbe Lage, wie das Band mit den Columellarknöchelchen bei Pelobates hat, für die Columella zu halten. Auf diese Weise erscheint die gewöhnliche Behauptung, dass bei den Urodelen die Columella gar nicht existirt, für Siredon und einige andere Formen, irrthümlich."

Iwanzoff seems, therefore, to have seen two structures, though their detailed relations were confused. The early description of Windischmann portrays most closely the condition in Ambystoma, though all the descriptions were doubtless approximately correct if the age (larval or adult) is considered. *No one seems to have recognized the fact that the condition changes with age.*

Turning now to the development of the "sound-transmitting" apparatus in Ambystoma, we find that only imperfect glimpses of the true development were obtained, and it is not always easy to determine to which structure they refer. Wiedersheim, Parker, Stöhr, Killian, Witebsky, Winslow, have examined one or more stages in the larval Ambystoma or Siredon (Axolotl). Wiedersheim and Winslow evidently described the development of the columella; Witebsky saw the independent chondrification of the columella but missed the connection of the stilus with the squamosum (or palatoquadrate) and drew from his observations some quite irrelevant conclusions as to the homology of the columella for which he had no sufficient basis in fact, as has been pointed out by Gaupp ('98) from whose paper our information is gained, Witebsky's dissertation being inaccessible to us here. Stöhr made his examination of the development in the Axolotl only incidental to a more detailed study of the development in Triton, so that he simply "confirmed" the results of Parker's more extended investigation, stating that the "operculum" grows out of the cartilaginous border of the fenestra vestibuli. He gives no figures, so that it is difficult to interpret his results. It was apparently the *Columella* (our use) that he had under observation. Parker alone examined an extended series of larval Ambystoma (Axolotl, Siredon) including an adult Ambystoma. For the first seven stages neither his words nor his figures give indubitable proof that he saw the development of the columella or recognized the stilus or its connection. His description (p. 564) of the adult (Ambystoma opacum) indicates that in his stapes he described the operculum.[6] In two points there was probably mistaken identification; ligament and ossification were doubtless absent. The descriptions of two large Axolotls (8½ and 8¼ inches long) undoubtedly applied to the Columella,[7] the recognition of the suspensorial connection above the facial nerve being noteworthy.

[6]"The bulging bony floor of the vestibule forms a widely crescentic bulla, and in the notch the fenestra ovalis contains a small lenticular stapes, the center only of which is ossified. The spiracular ligament fastens the stapes to the back of the top of the suspensorium."

[7]Page 559, "the stapes (Figs. 2, 4 and 5, st.) is unusually solid and projecting, its outstanding process looking a little forward. From that process

Winslow ('98), on the other hand, has described and figured models of three stages of the chondrocranium of Ambystoma. He describes the columella (Stapes) chondrifying as an independent center in a 12 mm. individual. The condition in a 37 mm. larva he describes as follows: "The fenestra ovalis is now nearly filled by the stapes (s), which has a slight prominence directed outwards and upwards from the antero-dorsal angle of the cartilage towards the otic process of the quadrate. These cartilages, however, do not become united at any time during the development of Ambystoma as they do in some other forms." In a young adult, 69 mm. in length, he describes the stapes as in the posterior portion of the fenestra closely applied against the remnant of the cartilaginous wall of the capsule, this time describing the operculum as stapes. His figure bears out the brief description and shows the stilus of the fused columella extending from the ear capsule to the palatoquadrate.

It is hardly necessary to comment on the difficulties of interpretation in such a form as Ambystoma if the changes at transformation are ignored. Transformation occurs relatively quickly and at this time the salamanders are less easily procured.

Chondrotus tenebrosus. Three examples of this species were examined; larval, transforming, and adult. It was not surprising to find that the relations in this salamander closely resembled those in Ambystoma because of the close relationships of the two genera.

The larva was well advanced (58 mm. long) and the morphological relations diagrammatic in their clearness. The otic capsule as also the remainder of the chondrocranium is unusually heavy, and the columella shares this characteristic. As shown in Fig. 34 (Pl. III) there is a well developed stilus which articulates closely with the lower edge of the squamosum. In no other form examined by us has a single section shown the distal connection as well as the central origin of the stilus from the fenestral plate, as a result of

a ligament arises which spreads into a fan-like fascia, which is inserted along the under and outer edges of the suspensorium from the lobe of the otic process to the lobe of the quadrate . . . it lies some height above the portio dura. . . ." Page 562. "Thus we have here what may be called a *spiracular fascia*, the counterpart of the spiracular cartilage and bone of the Menopome and others."

its more transverse course, since in Ambystoma as well as the other forms examined it has a course outward, upward and also forward (See, however, Siren, p. 598). The fenestral plate is at no point connected with the otic capsule, at the edge of the fenestra or elsewhere. A few sections farther forward the distal end of the stilus is joined by connective tissue with the caudal edge of the palatoquadrate, this junction not being as close, however, as that with the squamosum. The relations of artery, vein and facial nerve are as in Ambystoma (Pl. III, Fig. 34).

The second specimen was well along in transformation (150 mm. long, gills mere stumps). At this stage the columella is connected by membrane with the lips of the fenestra everywhere save on its cephalo-ventral border where cartilaginous fusion has begun. Its stilus is massive and abuts against the palatoquadrate with which it is joined by connective tissue (Pl. IV, Fig. 36, St. C.). The *Os. pterygoideum* also comes into close relation with it, but the connection with the squamosum existing in the larva is now much less direct (Fig. 36). The characteristic inner and outer ossifications are present, with accompanying changes in the cartilage.

The floor of the ear capsule medial to the caudal portion of the fenestra is nearly completely cut out as the operculum (Pl. IV, Fig. 38, *Op*). In its present development, it may be compared with the stage in Ambystoma shown in Fig. 32 (Pl. III). An opercular muscle attaches to the operculum. The recessus perilymphaticus is characteristically present.

The adult individual was 240 mm. long and was examined by dissection. The condition found was much like that in the adult Ambystoma. The columella, with its inner and outer bony surfaces, the latter bearing a well ossified stilus, is completely fused with the otic capsule forming the anterior boundary of the nearly circular fenestra into which was fitted a cartilaginous operculum, roughly hemispherical in form, its flattened surface bearing a tendon, doubtless that of the M. opercularis, which was not, however, dissected out. Fusion with the otic capsule was apparently by cartilage only, so that, by the use of some force, the stilus and plate could be and were completely broken away. The distal end of the stilus was con-

nected by syndesmosis with the under surface of the squamosal near its caudal edge, and doubtless also with the palatoquadrate cartilage as well, though this relation was not determined.

Examination of the condition in the adult of this salamander by means of dissection was made partly because other adult members of this family had been examined in this way. Of three Asiatic genera, Hynobius (Ellipsoglossa nævius) Salamandrella (kaiserlingi), and Ranidens (Randon sibericus) the "operculum" (columella) is described by Wiedersheim, '77, (503, 519) as a remarkably large cone of bone whose columella [stilus columellæ] is closely joined to the lower end of the quadrate. As a result of this intimate connection Wiedersheim called attention to the inevitable shaking of the perilymph that must attend every violent closing of the jaw,—as in the seizing of prey,—an arrangement whose physiological significance it is difficult to comprehend. Granting, however, that as in Ambystoma and Chondrotus, the columellar plate is fused with the ear capsule, the difficulty found in the close junction of stilus and palatoquadrate disappears, a fused element not being subject to violent agitation from movement of the jaw, but on the contrary offering a firmer support for the upper end of the quadrate.

The suggestion is, therefore, made that the columella will be found fused in the other Ambystomidæ, including these Asiatic forms, and it is not without some direct evidence. Fig. 65 in Wiedersheim's monograph ('77) shows the stilus in Ellipsoglossa (Hynobius) arising, not from the opercular plate, but from the anterior lip of the fenestra, as in Salamandra. This is also just as he figures it in Ambystoma Weismanni (tigrinum) ('79, Fig. 8),—quite different, as might be expected, from the relations in the Axolotl ('79, Fig. 12). The operculum had been removed. His ('77) Fig. 67 (Ranidens), however, suggests a condition such as is found in Cryptobranchus.

Okajima ('08) has recently given a short description of the columella in another member of the family,—Onychodactylus japonicus. An ossified plate, "hollow" in the center with cartilaginous border, he states (p. 353, 354) fits into a lateral fenestra vestibuli. A cartilaginous stilus is connected with the cartilaginous portion of the

quadrate. This is evidently the columella; an operculum was not recognized. A text figure of a section through the ear capsule recalls strongly the similar section in just transformed Chondrotus (Pl. IV, Fig. 38), already described.

Salamandra.

Salamandra maculosa has proved to be a form of rather unusual interest with regard to its sound-transmitting apparatus both in respect to the elements present and their relations when considered in the light of conditions in other forms.

The skeletal elements of the head of Salamandra are quite fully ossified. The os quadratum is composed of a thin outer shell of bone very closely associated with the under surface of the superimposed squamosum. Underneath the os quadratum is the palatoquadrate which is continued into the basal, otic and trabecular (ascending) processes. As has been pointed out by Wiedersheim ('77), the inclination of the supensorium to the long axis of the skull is almost transverse, a condition not met with in many salamanders. The inclination of these elements, whatever its cause, undoubtedly influences the nature of the suspensorio-columellar connection in all forms.

Both columella and operculum are present in the adult. The columella is a plate of partly calcified cartilage fused with the lips of the fenestra vestibuli except in the caudal third of its circumference where it forms the cephalic margin of the definitive fenestra. Fig. 40 (Pl. V) is a photograph of a section through the cephalic portion of the columella and shows how completely it is fused with the ear capsule in this region. In the same figure is shown the short and thick stilus which projects from the dorso-lateral aspect of the columella. It is composed of hyalin cartilage distally continuous with the cartilage of the palatoquadrate; proximally it passes over into the plate of the columella whose cartilage, calcified where it joins the ear capsule, may also be seen in this figure. To the squamosum it is joined by connective tissue representing a short ligament. Describing the "stapes" in Salamandra, Parker ('82a) observes:—(pp. 174-5) "There is a well ossified lip, like the mouth of a pitcher,

to the fenestra ovalis: it looks outwards and backwards; the thick, oval closely fitting stapes (operculum) remains cartilaginous and is only attached to the supensorium by ligaments." In a foot-note he adds: "Mr. A. Doran has shown me a stapes, said to belong to this species, which is ossified and has a stalk. I find nothing of the kind in the specimen dissected by me; and I think it probable that that specimen (in the Hunterian museum) belonged to another kind." It is quite likely that Mr. Doran was correct and that the stilus had by some accident been torn from Parker's specimen. The relation of the stilus to the artery, vein and facial nerve is typical.

The operculum occupies the typical position below and behind the columella filling the definitive fenestra vestibuli. It is a relatively large hemispherical mass of cartilage free all round, attached to the lips of the fenestra by membrane only. The cephalic fourth is overlapped by the caudal margin of the columella precisely as in Ambystoma. This is made clearer by an examination of Fig. 41 (Pl. V), a photograph showing the operculum internal to an overhanging lip of cartilage, the columella, which conceals the entire cephalic and a portion of the dorsal margin of the operculum. In tracing it forward, it gradually diminishes and finally disappears in the middle region of the perilymphatic prominence. The photograph reproduced in Fig. 42 (Pl. V) is of a section in this region, and shows a small lip of cartilage, a backward continuation of the columella, projecting from the ear capsule upon the dorsal margin of the fenestra. Fig. 65 (Pl. X) is a schematic representation of the position and relations of the columella and operculum as viewed from the side.

The perilymphatic prominence is pronounced. To the caudal surface of the operculum is attached the strong M. opercularis which extends caudad to the suprascapula.

Wiedersheim ('77, p. 504), in commenting upon the columella in Salamandra, says: "It is cartilaginous throughout life and rests suspended by the connective tissue in the foramen ovale which is bordered by two thick, crest-like lips. These lips now pass forward and outward in a thin cartilaginous process and this joins the quadrate cartilage,—a remarkable variation of the relations described

above." We are able to confirm these early observations and in the light of the changes which take place in Ambystoma at transformation, offer an interpretation of the presence of the fourth process connecting the palatoquadrate with the ear capsule. Undoubtedly as in Ambystoma the columella becomes fused with the margin of the fenestra and maintains its connection with the suspensorium through the stilus which is without doubt the cartilaginous process from the fenestral lip to the quadrate cartilage seen by Wiedersheim.

We feel no hesitation in offering this interpretation of the structures in this form even though developmental stages have not been studied by us. The sound-transmitting apparatus in Salamandra is in all essential respects identical with that of Ambystoma in which a very large number of developmentad stages have been studied carefully, permitting an interpretation of the adult condition which without them would be difficult. Fuchs ('07) has figured and described the chondrification of the otic capsule in Salamandra, giving in some detail the formation of the fenestra vestibuli and operculum. Employing the nomenclature of Gaupp, he describes the operculum as formed chiefly by a cutting out of the already chondrified ear capsule and partly by the chondrification of the chondroblastema filling in the fenestra. The latter he recognizes existent from the beginning as an unchondrified portion of the otic capsule. The development of the *Operculum* of Salamandra he undoubtedly gives correctly and in the figures of models of three stages shows it forming upon the medial and caudal sides of the fenestra in the manner stated above just as the operculum is formed in Ambystoma and in Triton presently to be described. He neither discusses nor shows in his figures, however, the process described by Wiedersheim, the detailed development of which in this form would be of particular interest. No hint is given of its origin or relations except in Fig. 3 on page 11 where a slight projection from the dorsal boundary of the fenestra appears to be what we are inclined to interpret as the caudal tip of the columella. A comparison might also be made between his Figs. 3, I, and our Figs. 1 and 23 (Pl. I) of Ambystoma and Fig. 27 (Pl. II) of Triton.

Fuchs gives no intimation of the ages or sizes of the larvæ or

embryos studied. His theoretical conclusions drawn from the development of the operculum in Salamandra will be referred to in the second part of the paper.

Triton and Diemictylus.

In Cope's family of the Pleurodelidæ, the condition of reduction and incorporation,—if we may so state it,—is carried farther than in the form just described,—Salamandra. The connection with the squamosum and palatoquadratum is absent, and the fenestral plate forms the portion of the ear capsule bounding the cephalic part of the fenestra vestibuli above and in front. In the adult, the identity is entirely lost and it would be impossible to recognize the true morphological relations were it not for the condition in the larva. In fact, at the time of our first contribution, the existence of the columella in Diemictylus was not recognized even though larvæ had been examined. At that time we expressed confidence in its presence at some stage in the development of the larva, but thought that it was absorbed or became incorporated,—possibly with the operculum. It was, indeed, subsequently found to be present and incorporated not with the operculum, but with the edge of the fenestra. It was in the light of the conditions in Salamandra that the relations in Diemictylus became clear. The existence of the columella was first determined in a 19 mm. larva, the subsequent examination of Triton larvæ bringing confirmation. In this last form the significance of the morphological relations is more apparent so that it may best be considered first.

Triton cristatus. Through the kindness of Professor Robert Wiedersheim, we were enabled to examine a series of specimens of this form, serial sections being prepared of larvæ 18, 20, 25, 34, 36, and 37 mm. in length. In the smallest individual at our disposal (18 mm.) both operculum and columella are present, of cartilage, the latter fused more or less completely with the crista semicircularis, hence it has been impossible to determine how early this structure appears; whether at any time its proton is as obviously outside the membrana fenestræ as it is in Ambystoma, or whether it undergoes chondrification separate and distinct from the edge of the primary

fenestra, later becoming fused with it. There is some evidence in our series that leads us to believe that it will be found to chondrify as a separate piece of cartilage:—(a) its cartilage differs in its staining from that composing the crista semicircularis, presenting the appearance of "younger" cartilage; (b) it lies outside what might be considered the ideal plane of the fenestra and apparently outside the fenestral membrane; (c) in some of the larvæ a small cleft is left between the crista semicircularis and the columella. This cleft is especially well marked in the 36 mm. larva (Fig. 48, Pl. VII), but present on one side only, being therefore variable in its extent and doubtless simply an indication of imperfect fusion. The columella as in Ambystoma fills in the anterior portion of the primitive fenestra in its dorsal part; if the fusion with the ventral lip occurs early, it is conceivable that the dorsal fusion may be delayed.

As to the mode of formation of the operculum, the evidence is indubitable; it is forming in the "floor" of the ear capsule as a chondrification in the "opercular tissue" (membrana fenestræ) in its caudal and medial portions. In the 18 and 20 mm. specimens, the operculum is already outlined, but broadly continuous on its medial and caudal sides with the cartilage of the floor, and were it modelled, would doubtless present the appearance shown in the model of a 20 mm. Triton tæniatus published by Gaupp ('05, p. 695, Fig. 350). In the older larvæ, save for the larger size of the operculum and its more complete separation from the floor of the otic capsule, conditions have not been essentially changed. A model was made of this region in a 34 mm. larva which is reproduced in Fig. 27 (Plate II). Here the operculum is shown forming in the caudal portion of the fenestra still rather broadly connected with the floor of the otic capsule caudally and medially. The rudimentary fenestral plate is shown fused with the anterior portion of the crista semicircularis. The closure of the anterior portion of the primary fenestra is also being accomplished by a growth of cartilage upon its ventral side. This figure showing the primitive fenestra and its contained cartilages may be directly compared with the stage in Ambystoma shown in Fig. 24, Pl. I, and its diagrammatic elucidation in Text Fig. 1.

Three photographs (Pl. VII, Figs. 48, 49 and 50) of sections from the 36 mm. larval Triton are submitted in illustration of what has been said above. Fig. 48 is through the ear capsule cephalad of the (secondary) fenestra at a point where the columella is fused with the ventral lip of the (primary) fenestra (cf. Fig. 27, Pl. II). It shows the cleft between the dorsal margin and the crista semicircularis whose existence has been mentioned above. It might also be compared with Figs. 28 and 29, Plate XV, of Gaupp's ('93) monograph upon the Chondrocranium of the Frog. Fig. 49 is five sections (75 microns) farther caudad through the columella and just ahead of the tip of the operculum, while Fig. 50 is still farther back (210 microns) behind the caudal end of the columella and through the operculum. The position of the artery and vein may be noted, in comparison with Figs. 8, 9 and 10 of Stöhr ('79). It may be remarked that in the oldest of the larval Tritons examined (37 mm.) the M. opercularis has not yet appeared nor has the operculum become completely separated off from the cartilaginous ear capsule.

By comparing these figures (Pl. VII, Figs. 48-50) and the two similar ones of a Diemictylus larva (Text Figs. 4-5) with the sections through the ear capsule of the larval and transforming Ambystoma (Pl. III, Figs. 31-32-33) the basis of the homologies for Triton becomes evident. In a comparison of Triton and Ambystoma the early appearance of the operculum in Triton is noteworthy. Its proton is already partially outlined at 18 mm. in length, and doubtless recognizable at a still earlier stage, while in Ambystoma its appearance comes only with the beginning of transformation, and in the larva it is a part of the otic capsule. The columella element, on the contrary, while already evident in the youngest Triton larva, becomes more prominent in the later larval stages; in Ambystoma it appears very early. The growth of the columellar plate in Triton during the larval period with an increasing distinctness of demarcation from the crista semicircularis suggests, it must be admitted, the interpretation of a differentiation out of "fenestral tissue" as the fenestra increases in extent. As has been said, the available material does not permit us to determine the early transformations

that take place in the ear capsule in Triton. The presence of a rudimentary columella seems clearly established. The problem of its phylogeny may be better discussed from a comparative view-point.

No trace of a stilus or connection with the squamosum or palatoquadrate was to be found unless, possibly, a scanty group of cells extending from the columella around the outer side of the vena petroso-lateralis was its representative.

In the adult Triton the conditions are much altered. The otic capsule is strongly ossified; the operculum being an oval plate of cartilage filling in the relatively small fenestra vestibuli and projecting back a short distance. On its outer side it gives attachment to a well developed M. opercularis; on its inner side is the cavum perilymphaticum and its backward extension outside the otic capsule is the recessus perilymphaticus. To the otic capsule behind and to the edge of the fenestra the operculum is attached by membrane only. The fenestral margin is osseous behind; dorsal and ventral cartilaginous lips join to form the cephalic border of the fenestra, the cephalic end of the operculum being slightly included. The columella is completely merged with the otic capsule, though doubtless the dorsal and cephalic cartilaginous margin of the fenestra, in part at least, represents it. Figs. 43 (Pl. V) and 44 (Pl. VI) show the relations, shape and cartilaginous consistency of the operculum. The latter figure may be compared with Fig. 42 (Pl. V) of Salamandra in illustration of the more massive character of the operculum in that form. Recurring again to the columella, in these two forms, Triton presents a condition of much greater reduction and incorporation in the absence (in the adult) of a recognizable fenestral plate and stilus columellæ (cf. Figs. 41 and 43, Pl. V).

The development of the otic capsule in Triton has been followed in more or less detail by Reichert '38, Semmer '72, Parker '82b, Wiedersheim '77, and Stöhr '79. From the reduced condition of the columella in this salamander, it was to be expected that its presence, not to mention significance, should be entirely overlooked and that the development of the operculum should be given correctly in all essential points. Hence we find Reichert, Semmer, Wiedersheim, Parker, and finally, Stöhr describing the operculum as formed

out of the otic capsule in this Urodele. Stöhr's classical investigation was undertaken primarily to describe whether or not after all the columella (of reptiles) was not of double origin,—one portion derived from the ear capsule (opercular plate) and a second portion from the hyoid arch, and as a preparatory study it seemed best to him to re-examine conditions in the amphibia (p. 480). Parker, in the meantime, published his first paper, dealing largely with the development of the skull of the Axolotl (to which reference has already been made, p. 562, but including a brief description of the skull of the larval and adult Salamandrina (Seironota) perspicillata. His second paper, dealing with Triton, appeared subsequently to Stöhr's. Wiedersheim ('77) had described (apparently in Triton alpestris) the development of the operculum as cut out of the otic capsule by a circular thinning of the cartilage, which by extension completely separates the operculum from the remainder of the otic capsule as a nearly circular disc.[8] With this mode of development as with Reichert's view that the operculum appeared as a chondrification in the fenestral membrane, Stöhr disagreed. While he examined, apparently, larvæ of different ages, he described, modelled and figured the condition in a 24 mm. specimen. He says: "Vom vorderen Rande des knorpeligen Fensterrahmens entspringt ein kurzer nach hinten gerichteter Fortsatz (O), der auf der häutigen Fenestra ovalis aufliegt. Zu beiden Seiten des Fortsatzes liegen grosse Blutgefässe (die Rinnen sind auf der Figur sichtbar), die bei der Vergrösserung desselben in so fern eine Rolle spielen mögen, als sie durch Druck die knorpeligen Rinnen, welchen sie anliegen, immer mehr vertiefen und schliesslich den Boden der Rinnen zum völligen Schwund bringen. Die Vergrösserung des Fortsatzes erfolgt aber gewiss auch durch eignes Wachstum. Bei Triton cristatus

[8] ('77, p. 501). "Kurz nach Verschmelzung der Parachordal-Elemente mit den Gehörblasen sieht man am äusseren Rand ihrer Unterfläche eine ringförmige Zone auftreten, welche bei genauerem Studium sich als eine circuläre Verdünnung der Knorpelwand herausstellt. Letztere schreitet immer weiter fort *und schliesslich hat sich eine rundlich-ovale Knorpelscheibe aus der Labyrinthwand* (Fig. 6, *Fov, Op*) *herausgeschnürt, ein deutlicher Beweis, dass das Operculum der Urodelen ontogenetisch nicht vom Kiemen-Apparat, sondern von der Gehörkapsel selbst herzuleiten ist.*"

sowohl, wie bei Triton tæniatus bleibt der Fortsatz nicht lange mit dem knorpeligen Fensterrahmen in knorpeliger Verbindung; ehe er noch eine Grösse erreicht, schnürt er sich von seinem Mutterboden ab und stellt nun ein freies, auf der Fenestra ovalis aufliegendes Knorpelplättchen dar; das Operculum. * * * *Das Operculum ist demnach ein Theil der knorpeligen Ohrkapsel, hervorgewachsen vom vordern Umfang des ovalen Fensters. Mit dem Hyoidbogen steht es genetisch in keiner Beziehung."* Interestingly enough, the description and figures indicate that the structure he identified as the Operculum was not the Operculum but the Columella. Its shape, connection with the anterior border of the fenestra, and its position between artery and vein, leave small doubt of this. The development of the *operculum* he seems to have entirely missed, and the mode of development advanced by Wiedersheim appears to be more nearly correct, though doubtless chondrification of opercular tissue in the fenestra vestibuli also contributes to its growth. It seems certain that in the growth of the larva both the fenestra and the operculum increase in size, and observation of this enlargement and extension of the rather cleft-like fenestra (*vide* Gaupp '05, Fig. 350) doubtless caused Parker to believe that the oval window arose by "dehiscence," and explains also the mode of origin of the operculum advanced by Semmer and by Wiedersheim. On the other hand, Stöhr's contention that the fenestra exists from the beginning as an unchondrified portion of the ear capsule, is without doubt true for Triton as we find it to be for Diemictylus, but describes the origin of the primary rather than the secondary fenestra. Stöhr, in a year-old Triton, figures the true operculum.

Parker's brief statements ('82b, pp. 198, 199, 203, 206, 209) upon the operculum (Stapes) and fenestra vestibuli (ovalis) in larval and adult Triton (Triton punctatus) are too indefinite to permit of any conclusions being based on them as to what the conditions were. In the adult he comments upon the cartilaginous nature of the operculum (Stapes) and the lack of a stilus (columella).

Diemictylus viridescens. Little need be said of the conditions in this form since in all essentials it resembles Triton closely. In the transformed salamander (red form) as also in the fully adult (Figs.

2-3), the operculum is plate-like, of cartilage, having the same relations to the fenestral margin as in Triton. It is somewhat more massive; the fenestral margin is cartilaginous only in front and above where the cartilage extends back to the caudal end of the fenestra. Below, cartilage extends back only as far as the anterior tip of the operculum. A well developed M. opercularis is present.

During the larval stage the morphological relations are essentially those of Triton larvæ, the vestigial plate being somewhat more closely joined to the crista semicircularis (Fig. 4, *Cr. s.*), but its

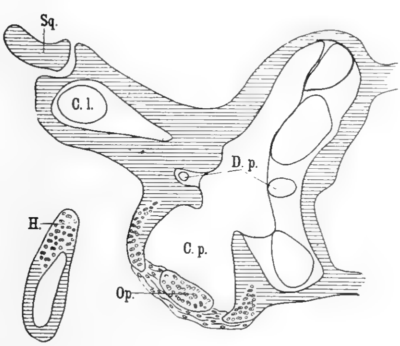


Fig. 2.

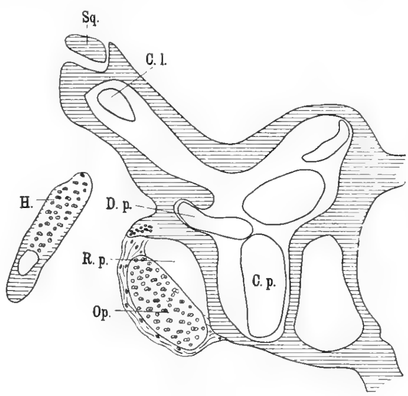


Fig. 3.

Fig. 2. Diemictylus viridescens, land form. *C.l.*, canalis lateralis; *C.p.*, cavum perilymphaticum; *D.p.*, ductus perilymphaticus; *H.*, ceratohyale; *Op.*, operculum; *Sq.*, os squamosum.

Fig. 3. Diemictylus viridescens, land form. *C.l.*, canalis lateralis; *C.p.*, cavum perilymphaticum; *D.p.*, ductus perilymphaticus; *H.*, ceratohyale; *Op.*, operculum; *R.p.*, recessus perilymphaticus; *Sq.*, os squamosum.

ventral edge more evidently outside the membrana fenestræ. At 15 mm. in length, neither columella nor operculum have appeared. In a 17 mm. specimen, the columella is developing upon the fenestral membrane in continuity with the fenestral margin above and in front. The operculum is forming on the medial edge of the fenestra in continuity with the cartilage of the capsule. Aside from growth and a more marked differentiation of the operculum, there has been but slight change in a larva 19 mm. long. At 37 mm. the operculum and also the columellar plate are well developed. The relations

of the two structures may be seen from the accompanying figures, 4-5.

These figures may be compared with the similar figures of the 36 mm. Triton larva (Pl. VII, Figs. 49 and 50). Fig. 4 may also be compared with Fig. 41, Plate V, of Salamandra and Fig. 32, Plate III, of transforming Ambystoma.

Adequate descriptions of the "sound-transmitting apparatus" in Diemictylus are lacking. Parker ('82a) describes the condition in both larva and adult (pp. 179, 181), but each statement is brief and without significance; more significant is his comment when

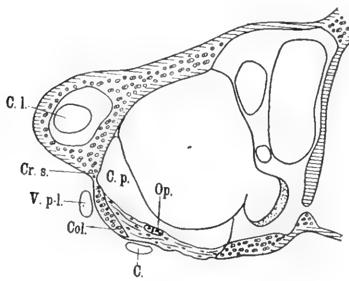


Fig. 4.

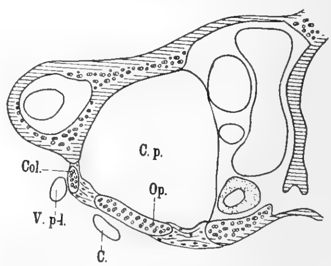


Fig. 5.

Fig. 4. Diemictylus viridescens, larva 37 mm. long. *C.*, arteria carotis interna; *C.l.*, canalis lateralis; *Col.*, columella; *C.p.*, cavum perilymphaticum; *Cr.s.*, crista semicircularis; *Op.*, operculum; *V.p-l.*, vena petroso-lateralis.

Fig. 5. Diemictylus viridescens, larva 37 mm. long. *C.*, arteria carotis interna; *Col.*, columella; *C.p.*, cavum perilymphaticum; *Op.*, operculum; *V.p-l.*, vena petroso-lateralis.

characterizing the skull of Spelerpes (See foot-note on p. 578). Cope ('88) is not sufficiently detailed and Wiedersheim ('77) does not particularly emphasize the relations in this portion of the skull of Diemictylus.

Plethodontidae.

Representatives of all the genera of this family have been examined as follows: Batracoseps, Hemidactylium, Manculus, and Autodax, the adult stage only; Gyrinophilus, Plethodon, and Stereochilus, both larva and adult; Spelerpes, adult and a series of twelve developmental stages of larvæ from 15 to 55 mm. in length.

In the fenestra vestibuli of the adult there is a single plate, irregularly oval in outline and free from the ear capsule except on its ventro-cephalic margin where a fusion is found (Pl. X, Fig. 67). From the cephalic and dorsal portion of this plate there projects upwards and forwards a slender stilus (Fig. 67) which in the adult articulates with both quadrate and squamosum. It is slender in all forms except Batracoseps where it is absent or vestigial. In the caudal portion, the fenestral plate extends behind the caudal margin of the fenestra and swells outward, forming a prominence in this region. The cavity of this prominence is an outward and backward extension of the cavum perilymphaticum. In a relatively deep depression on the caudo-lateral aspect of this prominence the M. opercularis is attached. The fenestral plate in the Plethodontidæ is, as are all the related parts, finer and much less massive than in the families already considered. The stilus is relatively a long and slender rod between the vena petroso-lateralis above and the arteria carotis interna and facial nerve below. The lips of the fenestra vestibuli remain cartilaginous and are connected with the fenestral plate by membrane only, save in the cephalo-ventral portion mentioned above.

The inner and outer bony plates so characteristic of the columella in other forms are here co-ossified, the cartilage persisting only at the circumference. In the central portion of the plate the cartilage which is subsequently replaced by bone, varies markedly in thickness even in the same genus,—for example, in Spelerpes bislineatus it is small in amount and early replaced by bone, while in Sperlerpes ruber quite the reverse is true. A similar comparison could be made between genera. The ossification of the fenestral structures in this family corresponds to the ossification of the skull as a whole, both in time and extent.

Accompanying this marked ossification of the plate there is complete ossification of the base of the stilus; distally it is composed of a shell of bone enclosing a cartilaginous core. In regard to the relation of the peripheral end of the stilus, it should be stated that the articulation with the squamosum, quadrate and palatoquadrate mentioned above applies only to the adult. In the larvæ of the forms

studied (Gyrinophilus, Plethodon, Stereochilus, Spelerpes) the connection was with the squamosum only. In the adult there is a very wide variation, from a close articulation with the squamosum only (as in Manculus) to an intimate connection of the stilus with a cartilaginous process of the palatoquadrate, as in Stereochilus, Autodax and Plethodon. The other genera show an intermediate condition; in Hemidactylium and Spelerpes the connection with the squamosum is the closest, while in Gyrinophilus there is an equally close articulation with the subsquamosal process of the quadrate; in no one of them was the cartilaginous process of the palatoquadrate as well developed as in Stereochilus, Autodax, and especially Plethodon.

The variation in the connections of the distal end of the stilus is undoubtedly associated with the inclination of the suspensorium to the long axis of the skull. During the period of growth, due to the relative displacement of parts, the distal end of the suspensorium is "drawn" forward, bringing its long axis more nearly parallel with that of the skull.

The definitive fenestra vestibuli of the Plethodontidæ is large and lateral in position, more nearly representing the primary fenestra of other urodeles,—a condition recognized by Parker.[9] The columella is correspondingly large, nicely filling the opening, and projecting slightly back of it, as presently to be described. The plan of ossification in the columella is so characteristic that we have come to consider it as the Plethodontid type.

The M. opercularis, though absent in the larva, is a well developed structure in the adult. It attaches to the caudal portion of the fenestral plate, which there possesses a decided excavation occupied by the tendon of the muscle, giving the "scooped out" appearance noted by Parker.

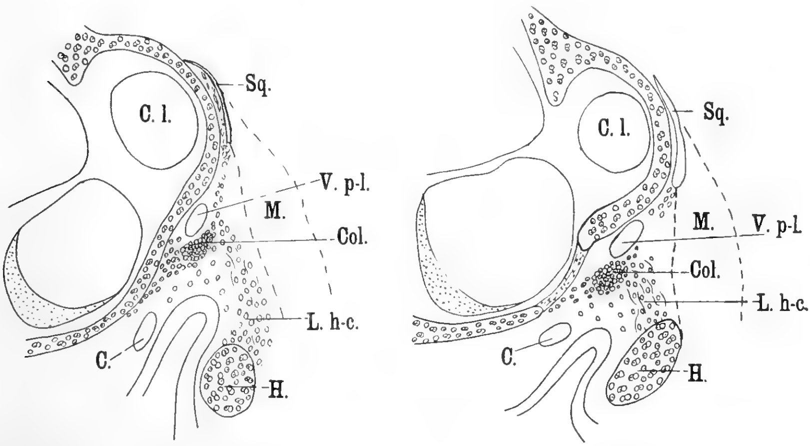
[9]Parker, '82, p. 199. "The under face of this ear capsule (in Spelerpes) is very different from that of most high "Urodelous" skulls.

"In those massive *typical* Caducibranch skulls just described, *e. g. Notophthalmus* (Pl. XVII) and *Cynops* (Pl. XVIII), the vestibule is in the form of a smooth *bulla*, with the stapes set on behind; and that plate is either quite soft or very slightly ossified; it is also relatively small.

"In *Spelerpes* (Pl. XXI, Figs. 2, 3, and Pl. XVIII, Fig. 10) the vestibule is but little protuberant; its fenestra is lateral, and corresponding with the stapes, very large."

Despite the depression for the M. opercularis the ear capsule has in this region a bulging appearance due to the growth of the fenestral plate backwards beyond the margin of the fenestra vestibuli. Fig. 47 (Pl. VI) shows not only that the prominence has extended beyond the margin of the fenestra, but also that within the prominence there is a recessus which is continuous with the cavum perilymphaticum.

Earlier in this paper it has been shown that in Ambystoma and others there is a perilymphatic prominence in the region of the



FIGS. 6 and 7. Plethodon cinereus, embryo. *C.*, arteria carotis interna; *C.l.*, canalis lateralis; *Col.*, columella; *H.*, ceratohyale; *L.h-c.*, ligamentum hyo-columellare; *M.*, musculus cephalo-dorso-mandibularis. *Sp.*, os squamosum; *V.p-l.*, vena petroso-lateralis.

fenestra vestibuli formed by the outward and backward growth of the operculum and that to the lateral aspect of this structure the M. opercularis is attached. Within the perilymphatic prominence is the recessus perilymphaticus, a caudal continuation of the perilymphatic cavity. In these respects there is a striking similarity between the caudal portion of the fenestral plate in the Plethodontidæ and the operculum of other forms. Further than the similarities mentioned in the preceding paragraph there is, in the adult, no evidence as to the constitution of the fenestral plate in the Plethodontidæ. In the light of development, however, the condition is

somewhat better understood. Eleven larvæ of Spelerpes bislineatus ranging in length from 15 to 55 mm. were studied by means of serial sections. Just when the columella makes its appearance we can not say, but in larvæ 15 and 17 mm. long there is present a delicate cord of cells outside the ear capsule extending from the under side of the squamosum towards the fenestral membrane to which it is not closely related at this stage. In a larval Plethodon cinereus (Figs. 6 and 7) of a similar stage of development this cord is composed of a greater number of cells and is consequently more com-

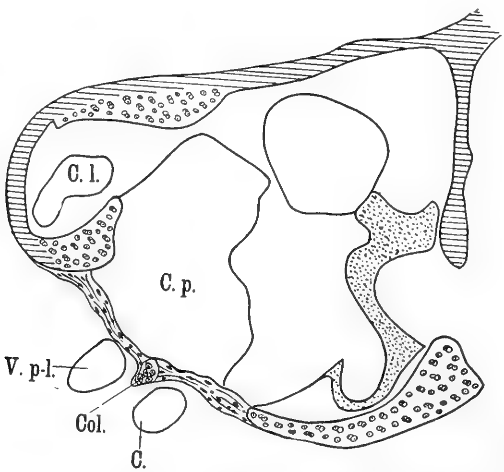


Fig. 8. Spelerpes bislineatus, larva 23 mm. long. *C.*, arteria carotis interna; *C.l.*, canalis lateralis; *Col.*, columella; *C.p.*, cavum perilymphaticum; *V.p-l.*, vena petroso-lateralis.

pact and larger. Here also, as in Spelerpes, this group of cells is not closely connected with the fenestral membrane. It extends forward to the under side of the squamosum and apparently is joined to the ceratohyal by a less marked cord of cells. Figs. 8 and 9 from two sections 25 microns apart may serve to illustrate the extra-otic position of the columella. Comparison with the figures of like stages of Ambystoma and Cryptobranchus is suggested.

In Sperlerpes larvæ 23 mm. long the lower end of this cord of cells has become intimately associated with the fenestral membrane at the cephalo-ventral margin of the fenestra. At this point the fenes-

tral plate remains permanently fused with the ear capsule. Chondrification has occurred, forming a stilus and a small fenestral plate (Figs. 8 and 10).

From the place of its attachment the fenestral plate gradually extends backwards, growth being the result of the deposition of cartilage in the free margins of the plate, particularly the caudal. Thus successive rings are added until the plate fills the opening in the ear capsule. In the caudal portion of the fenestra vestibuli the mem-

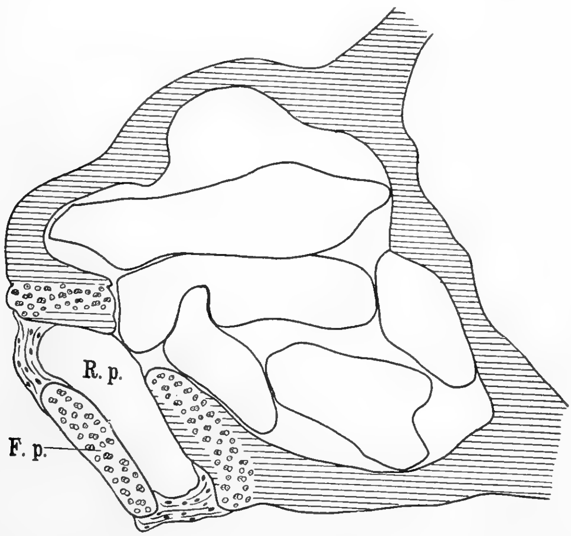


FIG. 9. Spelerpes ruber, larva 71 mm. long. *F.p.*, fenestral plate; *R.p.*, recessus perilymphaticus.

brane forms an outpocketing or prominence (Fig. 12) long before it is invaded by cartilage, but before transformation the extension of the prominence behind the lips of the fenestra is slight if present at all. The prominence forms the outer walls of a recessus which has the same relations as that of the adult. Until transformation there is no indication of the M. opercularis, it apparently having no function until adult life is assumed. The conditions in the adult Plethodontidæ are well illustrated by the photographs of Gyrinophilus in Figs. 45, 46, 47 (Pl. VI).

It appears that the definitive plate of the adult is the result of a direct and continuous growth of cartilage in the membrane which covers the foramen vestibuli. At no time during development are there found separate centers of chondrification in either stilus or fenestral plate.

There appears to be a wide divergence between the sound-transmitting apparatus in the Plethodontidæ and that in Ambystoma. This seems particularly true of the development, and as a consequence is also true of the homology of parts. A brief review of the development of the fenestral elements in the forms thus far considered may, therefore, render the situation easier to grasp. In Ambystoma the ear capsule chondrifies early and the columella fills the fenestra vestibuli. At transformation (there being no room for growth) the operculum is cut out from the cartilage of the ear capsule itself; an adaptation, it might seem, to mechanical needs. In Triton and Diemictylus the columella relatively early fuses completely with the

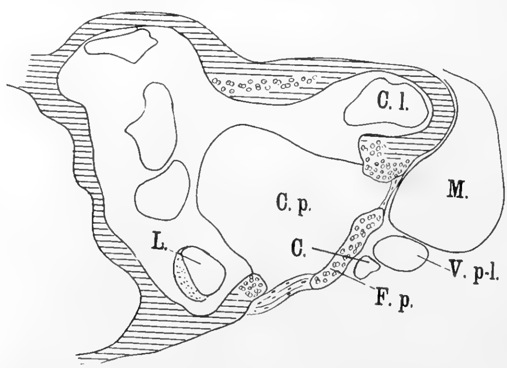
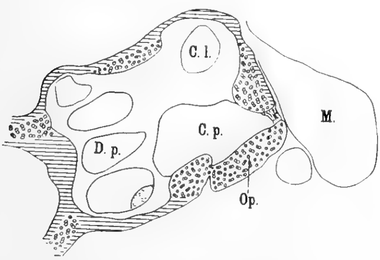


Fig. 10. Spelerpes bislineatus, larva 43 mm. long. *C.*, arteria carotis interna; *C.l.*, canalis lateralis; *C.p.*, cavum perilymphaticum; *F.p.*, fenestral plate; *L.*, lagena; *M.*, musculus cephalo-dorso-mandibularis.

ear capsule, leaving the foramen free. Here it is found that, while there is a slight cutting out of the operculum from the ear capsule, it results largely from a growth of the cartilage cephalad into the fenestral membrane. The situation in the Plethodontidæ differs from either of these. The columella, when first coming into intimate relation with the ear-capsule, is small as compared with the size of the for-

amen which increases in size with the growth of the skull. The early ossification of the ear capsule renders impossible the formation of an operculum by either the cutting out process or by forward growth into the fenestral membrane. A comparison of Figs. 11 and 12 will help to make clear that the membrane filling the caudal portion of the fenestra corresponds in its position and relation to other parts to that portion of the fenestra in Ambystoma which is occupied by the operculum. This, together with the formation of a prominence by the fenestral membrane in this region, which when chondrified affords attachment for the M. opercularis, suggests that it is opercular tissue. Taking this view it is perhaps not inappropriate to consider that the caudal portion of the fenestral plate in the Plethodontidæ represents the operculum of other forms.



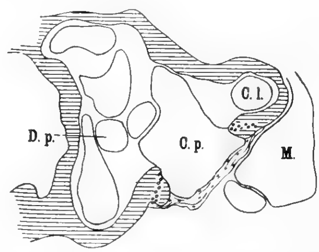


FIG. 11. Ambystoma punctatum, transforming larva. *C.l.*, canalis lateralis; *C.p.*, cavum perilymphaticum; *D.p.*, ductus perilymphaticus. *M.*, musculus cephalo-dorso-mandibularis; *Op.*, operculum.
FIG. 12. Spelerpes bislineatus, larva, 55 mm. long. Lettering as in Fig. 11.

This is from the viewpoint of groups in which the operculum is formed as a separate structure. Looking at it from the reverse point of view the Plethodontidæ might be considered as representing the less specialized condition. It is conceivable that in those forms in which an operculum is developed the columellar blastema became fused with the edge of the primitive fenestra both in front and behind, so that when it came to chondrification there were almost of necessity, developed two plates: (1) the fenestral plate of the columella joined to the otic capsule in front, and (2) the operculum joined to the ear capsule on the caudal and medial side. The operculum would thus be a dissociated part of the fenestral plate. In

the Plethodontidæ, due possibly to an early fusion of the columellar blastema in front only, such a division of the plate does not occur. It seems quite possible that a comparative study of the primitive fenestra in urodeles might afford some evidence of this view. What bearing this interpretation might have upon the periotic origin of the operculum is evident.

Desmognathidae.

Desmognathus fusca. In this species, the only member of the small family examined, the "sound-transmitting" apparatus mark-

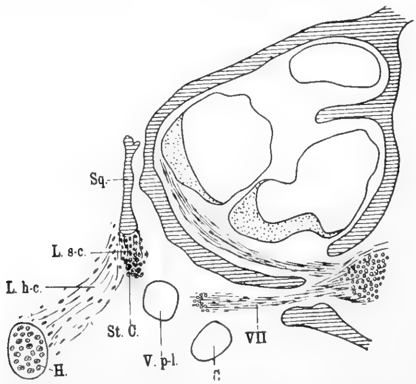


Fig. 13. Desmognathus fusca, larva 26 mm. long. *C.*, arteria carotis interna; *H.*, ceratohyale; *L.h-c.*, ligamentum hyo-columellare; *L.s-c.*, ligamentum squamoso-columellare; *Sp.*, os squamosum; *St.C.*, stilus columellæ; *V.p-l.*, vena petroso-lateralis; *VII.*, nervus facialis.

edly resembles in its general structure and relations that of the Plethodontidæ just described. In fact, they are of the same type. A bony plate with cartilaginous border fits into the fenestra and projects caudad upon the outer wall of the recessus perilymphaticus. The caudal portion is concave externally, and in the depression there is inserted the M. opercularis. The cephalo-ventral border is continuous with the cartilaginous border of the fenestra. The stilus is osseous with a cartilaginous core, the distal portion being of cartilage alone. Two figures of an adult, one (Fig. 15) through the caudal portion showing the concave fenestral plate and the recessus: the second (Fig. 14) through the insertion of the stilus, may be

directly compared with the sections through the same regions in the plethodontid, Gyrinophilus (Pl. VI, Figs. 46 and 47). Similar comparison may be made with Spelerpes (Figs. 6 and 7).

The relations of the stilus to the squamosum, quadrate, facial nerve, artery and vein, have been described in some detail by Kingsbury ('03, pp. 321-325) for larva and adult, two sizes of each, and as our investigations have not added significant details, it will be sufficient to state in confirmation of the description there given that while in the larva the stilus is most closely joined to the ventral edge of the squamosum (Fig. 13); in the adult it becomes shifted so as to articulate closely with the os quadratum, less closely with the cartilaginous process of the palatoquadrate. The relation to artery, vein and nerve is that typical of the majority of urodeles.

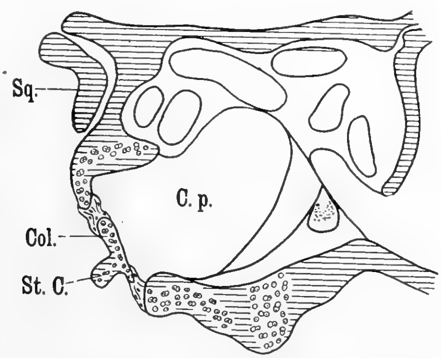


Fig. 14. Desmognathus fusca, adult. *Col.*, columella; *C.p.*, cavum perilymphaticum; *Sq.*, os squamosum; *St.C.*, stilus columellæ.

An examination of young larvæ and embryos which would determine the origin or origins of the stilus and fenestral plate, has not been undertaken. The mode of insertion of the stilus upon the fenestral plate, as shown in Fig. 14 (compare also Pl. VI, Fig. 46, Gyrinophilus), might suggest that the stilus alone developed outside the otic capsule, as the description of Parker would indicate. Since the columella in Desmognathus doubtless develops in essentially the same manner as in the Plethodontidæ (Spelerpes, Plethodon) reference may be made to the description of its development in that family, as given on p. 580.

Desmognathus possesses a short but well developed ligamentum hyo-columellare, in this respect also agreeing with many of the Plethodentidæ. In Fig. 13 it is indicated in the larva.

Typhlomolge.

A single adult 95 mm. long was studied by means of serial sections and a model of the entire skull in the otic region. The fenestra vestibuli is filled by a single plate as in the Plethodontidæ, connected by a long and slender stilus with the suspensorium. On the cephalo-ventral margin the fenestral plate is fused with the ear capsule.

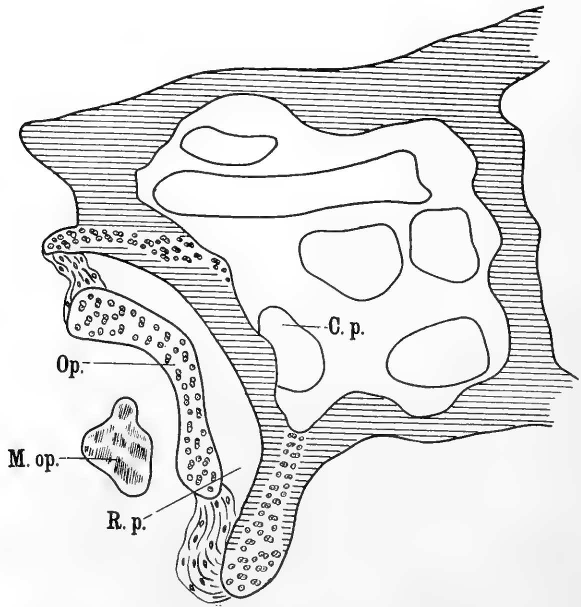


Fig. 15. Desmognathus fusca, adult. *C.p.*, cavum perilymphaticum; *M.op.*, musculus opercularis; *Op.*, operculum; *R.p.*, Recessus perilymphaticus.

The stilus is relatively longer than in any of the other forms examined. This is probably due to the extreme flattening of the skull, which has brought the long axis of the suspensorium more nearly parallel with that of the skull, thus throwing the attachment of the stilus to the suspensorium farther cephalad. It seems likely that this flat condition of the head is the result of an adaptation to sub-

terranean life where a wedge-shaped form is more convenient for making progress in narrow quarters.

Miss Ellen Tucker Emerson, 2d ('05), states in substance that the operculum is not connected with the suspensorium, and her figures show a short stilus, labelled columella, projecting freely from a basal portion in the fenestra vestibuli called operculum. Serial sections and the model of the specimen studied by us show that the columella is morphologically connected with the suspensorium but interrupted. On the right side of our specimen the stilus begins to grow smaller in diameter a litle beyond the middle of its extent and finally disappears for a distance of 210 microns. Then it appears again, gradually assuming its normal diameter, and continues uninterruptedly to the suspensorium. Here it is joined to the squamosum, the os quadratum and the palato-quadrate (Pl. II, Fig. 28). Between the free ends of the segments is a well defined ligament within which a few cartilage cells are found at intervals.

On the left side there are three segments (Pl. X, Fig. 68) produced by two interruptions. One of these corresponds in position and extent with the single interruption on the right side. The second occurs just before the stilus joins the suspensorium and is about the same in extent as the first. On this side the distal or third segment extends for a distance along the ventral edge of the squamosal, joining the edge of the os quadratum as well, but without relation to the palato-quadrate. It should be noted in this connection that Kingsbury ('03) described the stilus in adult Spelerpes as connected with a rod of cartilage lying along the ventral edge of the squamosum, a condition similar to what is found on the left side of our specimen of Typhlomolge.

It may be questioned whether the interrupted condition of the stilus in Typhlomolge results from tension and a consequent separation of what otherwise would remain, as developed, a continuous bar of cartilage, or is reminiscent of the condition found in the frog where there are separate centers of chondrification. It should be recalled, however, that in the urodeles where development has been studied, chondrification of the stilus and columella is continuous. Furthermore it is to be noted that in the Plethodontidæ there is a

loose connection of the stilus with the suspensorium, probably due to the tilting of the latter in its relation to the long axis of the skull. In Typhlomolge this tilting of the suspensorium is extreme and it is, therefore, a fair question as to whether or not the segmentation in the stilus is the result of tension during development. The attachment of the stilus to both suspensorium and columella are at the same horizontal level, there being a slight outward and downward curvature as shown in Plate III, Fig. 28.

The relation of the stilus to the blood vessels and nerves in this region is that found in Necturus, namely, the vena petroso-lateralis and the ramus jugularis of the facial nerve are above, while the main trunk of the facial nerve and the internal carotid artery are below. This condition is illustrated by Fig. 28 (Pl. III) and Text-figure 16.

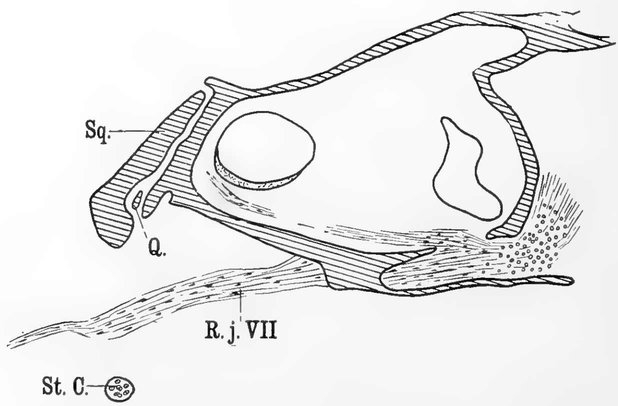


Fig. 16. Typhlomolge, adult. *Q.*, os quadratum; *R.j.VII.*, Ramus jugularis VII.; *St. C.*, stilus columella; *Sq.*, os squamosum.

The fenestra vestibuli is irregularly elliptic in outline as is the fenestral plate. The dorsal and ventral portions are not filled by the plate, there being a relatively wide space both above and below filled only by membrane. In the caudal portion there is a slight outward swelling forming a prominence which projects slightly beyond the caudal lip of the fenestra and which contains a small but well defined recessus within. The M. opercularis is absent.

The lips, both of the fenestra vestibuli and the fenestral plate,

remain cartilaginous throughout the greater part of their extent, the bony portion of the plate being much thinner than the margin as in the Plethodontidæ. At its base the stilus is composed entirely of bone (Fig. 17) which gradually becomes a sheath surrounding a core of cartilage. In the distal half (Fig. 16) the stilus is composed of cartilage alone.

Between the distal end of the ceratohyal and the otic region of the skull is a wide sheet of fascia, the caudal portion of which is attached to the stilus and in this region becomes a relatively strong ligament between the columella and hyoid (Pl. X, Fig. 68).

Developmental stages have not been studied, but it appears that in Typhlomolge the type of sound-transmitting apparatus is essentially that of the Plethodontidæ.

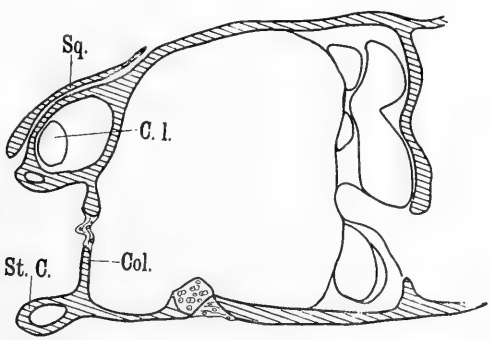


Fig. 17. Typhlomolge, adult. *C.l.*, canalis lateralis; *Col.*, columella; *St.C.*, stilus columellæ; *Sq.*, os squamosum.

Necturus.

Although the relations and development of the otic region of the skull in Necturus have been already quite well worked out by several persons, because of its historic interest in this connection and the general acceptance of its low systematic rank, it has been included for brief consideration. In illustration of the conditions in this form four photographs are introduced, Figs. 57, 58, 59 (Pl. VIII), and 60 (Pl. IX).

Miss Platt ('97), p. 430, described the early development in the ear capsule in Necturus. In a 19 mm. larva the "operculum" is

pre-cartilage, forming neither from the otic capsule nor the fenestral membrane, but independently lying primarily anterior to the fenestra. She suggested at this time the possible homology of the urodele operculum with the pars interna plectri which is supported in this paper; but evidently without much faith in its correctness, for in a footnote in which she states her objection to the term "operculum" she adds that this cartilage (operculum, *i. e.*, columella) is not the equivalent of the "columella" (plectrum) of the Anura and is of questionable homology with the "stapes" of higher vertebrates.

Kingsbury ('03) examined the early development in Necturus. He confirmed Miss Platt's results and in addition described the primary connection with the squamosum and the cells underlying it: "At this stage, the operculum is just beginning to chondrify as a distinct center, and from it a chord of cells is continued forward, ventral to the vena jugularis and the ramus jugularis, to the cells surrounding the developing squamosum, becoming continuous with them a short distance (50 microns) back of the processus oticus quadrati. The cells are of course continuous with those of the squamosum and also with the cells between that bone and the quadratum, so that the squamosum, the quadratum, and the ligament-anlage, may be said to be joined together by a common mass of cells. In the just hatched larva, likewise, the ligament-anlage clearly goes to the underside of the squamosum and inserts itself between that bone and the processus oticus quadrati, so that it might be interpreted as going to both structures. As soon as the connective tissue fibers develop, however, the relation is seen to be with the squamosum and not with the quadratum. In later stages a strong ligamentum squamoso-columellare develops, which connects the stilus columellæ to the columellar process of the squamosum."

Wilder ('03) in a monograph upon the Skeletal System of Necturus takes, however, a different view. He regards the operculum (columella, our term) as a "detached portion of the otic capsule" basing his view upon the condition in a 44 mm. larva which was essentially the same as in a 26 mm. individual examined by him. In the light of the work by Stöhr on Triton and Siredon he regards the statements of Miss Platt as unfounded, admitting, however,

that the proton described by her might represent "a true columella or hyomandibular cartilage which may become fused with the true operculum," although he could find no trace of such a double origin. In comment on the above it may be said that to determine the first development it is necessary to go back of the condition in a 26 mm. larva as was done by Miss Platt. There is certainly no indication of a double origin, although the increase in size in the columella of Necturus, as in Ambystoma (p. 556), admits of two possibilities of interpretation. The development of the operculum as described by Stöhr in Triton and Siredon has been discussed on p. 573.

The ramus jugularis VII passes over the ligament (Pl. VIII, Fig. 58), as in Proteus and Typhlomolge described above.

The relations of the columella, squamosum and facial nerve in Necturus are well known through the work of Cope, Drüner, Kingsbury and Wilder, though the significance of the relations has not been adequately considered.

Huxley's classic paper on the skull of Necturus was in respect to his description of the relation of the columella, facial nerve, and suspensorium incorrect and misleading, occasioning a wrong idea of the urodele type, persistent even up to the present time. We find no trace of his ligamentum suspensorio-stapiale, over which the facial nerve passes. Operculum (our use) and M. opercularis are lacking.

Proteus agrees in all essentials with Necturus (Wiedersheim, Drüner, Kingsbury).

Cryptobranchidae.

Cryptobranchus allegheniensis. Through the generosity of Mr. B. G. Smith, of Syracuse University, who kindly sent us a series of embryo, larval and adult specimens, we were enabled to examine the relations in this interesting form with quite satisfactory results.

As the publications of Wiedersheim ('77), Parker ('82b) and Cope ('88) have shown, the Columella in the adult possesses a well developed stilus which articulates with the under (inner) surface of the squamosum. This relation is strikingly shown in the series of sections through the head of an adult 140 mm. long (Pl. VII,

Figs. 54-55; Pl. VIII, Fig. 56) which was at our disposal. The squamosum, furthermore, is the only skeletal element to which the columella is attached, save the os pterygoideum which works its way well up on the inner surface (Pl. VII, Fig. 54) of the palatoquadrate and whose upper edge is somewhat loosely connected with the end of the stilus columellæ. As though determined by the excessive flattening of the head in this form, the mandibular end of the palatoquadrate is well back and lateral, while the cranial attachment by means of the usual three processes is unusually far forward (cephalad). The whole suspensorium is therefore rotated from an "ideal" transverse position, and it would seem as though this displacement might well explain in a mechanical way (a) why the stilus columellæ joins the under surface of the squamosum instead of its caudal edge as in so many of the forms; (b) how it is possible for the os pterygoideum to come into such close relation to the columella; (c) the absence (in the *young* adult at least) of a connection of the stilus with the palatoquadrate or os quadratum.[10]

A second interesting feature found in this skull is the presence of what might be interpreted as a Ligamentum hyo-columellare (Pl. VII, Fig. 55, L. h. c.) which is essentially a thickening of the fascia covering the inner (under) surface of the M. cephalo-dorso-mandibularis. It extends from the ceratohyal to the outer surface of the stilus, extending with it to its attachment to the squamosum. As this ligamentous thickening of the fascia is relatively more marked in the larva, it will be mentioned again in connection with that stage.

The columella appears to ossify in the typical way; at the stage of our specimen it consists of a cartilaginous body (Pl. VII, Fig. 55; Pl. VIII, Fig. 56) with an inner and outer plate of perichondral bone, the latter extending out upon the stilus for a distance. The distal portion possesses no bony sheath and it was doubtless this that led Parker to distinguish here two elements, a *Stapes* (bony and

[10]From this bone as a center the ossification of the quadrate cartilage apparently proceeds. Kingsbury referred to it as "bone X," Gaupp has suggested tentatively the homology with the os quadrato-maxillare of Anura. Its final interpretation requires additional comparative investigation.

fitting into the fenestra) and articulated with a *Pharyngohyal,*[11] consisting of cartilage. This is, of course, incorrect, due to the inadequacy of the method of gross dissection, upon which he based his conclusions.

From the caudal edge of the palatoquadrate a rather delicate process of cartilage extends back to the ceratohyal to which it is applied, curving slightly over its upper surface. The name of Epihyal which Parker gave to this process of the quadrate is likewise certainly incorrect. Cope ('88) has termed this the "Hyosuspensorial cartilage;" it might more correctly be called *Processus hyoideus palatoquadrati,* since an *articulation* with the quadrate as described by him is not found. In other respects the description of Cope is essentially correct. Wiedersheim, likewise, describes the squamosal connection of the stilus and the position of the facial nerve correctly, though he apparently overlooked the condition here in the general portion of his paper.

Larval Cryptobranchus. Serial sections were made through the head of four Cryptobranchus larvæ whose lengths were, respectively, 28 mm., 34 mm., 45 mm., and one just hatched. Since in these series the morphological relations are in all essentials the same, it will be sufficient for the purposes of this paper to describe briefly the relations in the one which is of intermediate size (34 mm.), and supplement this with comments on some interesting features in the youngest,—the newly hatched larva.

The columella in this larva (34 mm.) consists of a roughly oval plate of cartilage resting upon the membrane closing the fenestra vestibuli. From the cartilaginous border of the fenestra the columella is separated by the membrane everywhere except at the cephalic edge where it articulates with the cartilaginous ear capsule at the crista semicircularis. In larvæ 28 mm. and 45 mm. long, the fenestral plate does not come into articular relation with the carti-

[11]Parker in his earlier paper ('76, pp. 559 and 587) referred to this cartilage as homologous with the elasmobranchian "Spiracular cartilage" and the annulus tympanicus of the Frog, the facial nerve passing below it. In the second paper, however, in which he terms it a pharyngohyal, the facial nerve is described as passing over it.

lage of the ear capsule, so that doubtless this is a variable feature. In the adult there appears to be a very slender cartilaginous connection. The portion of the fenestra vestibuli not occupied by the columella is thus a crescent of somewhat horse-shoe shape.

The stilus of the columella arises gradually from the cephalic half of the fenestral plate, passing at first more laterally and then in a curve cephalad and slightly dorsad to become closely attached to the lower edge of the squamosum. In its course it passes between the vein and artery, as is typical, the vein being above (Pl. VII, Fig. 51). The facial nerve is entirely ventral to it, though the jugular branch and ramus communicans IX come very close, the latter lying at first upon the dorsal side of the columella; it slips over the outer side, however, and joins the R. jugularis in gliding under the stilus just as the latter joins the squamosum.

The sheet of fascia covering the inner surface of the M. cephalo-dorso-mandibularis possesses two thickenings of morphological importance. The more dorsal of these (Ligamentum hyo-columellare) arises over the outer side of the ceratohyal, curves around its upper surface and passes forward to join the stilus columellæ at its bend and, as a sheath upon its outer side, accompanies the latter to its articulation with the squamosum. Fig. 51 (Pl. VII) illustrates the location of this ligament in its course from hyoid to columella and shows the muscle "bellying against" the ligament as a full sail against a rope. A portion of this seems to have been Parker's stapedio-suspensorial ligament. The more ventral and band-like ligamentous thickening passes from the ceratohyal to the caudal edge of the quadrate. It curves around this element and a portion of it joins the tendon of the M. cephalo-dorso-mandibularis at its insertion on the os angulare. This ligament must embody the ligamentum hyo-suspensoriale and as well the ligamentum hyo-mandibulare. In connection with the former, in the adult is developed the cartilaginous process of the palatoquadrate already described. Whether this process develops from an independent center or not, and at what stage it appears, cannot be determined from the material at hand.

In the larva, the suspensorium has not the "rotated" position that

it has in the adult, and the columella has no connection with the palatoquadrate, os pterygoideum or os quadratum. Furthermore, it is to be noted that it articulates with the lower edge of the squamosum and not with its under surface.

The newly hatched Cryptobranchus is of interest in this connection for two reasons: (a) the columella is represented by a condensation of cells from which a cord of cells passes cephalad (Pl. VII, Figs. 52-53) and dorsad to the group of cells in whose midst the squamosum is just appearing as a homogeneous scale of bone; (b) the columella at this stage is pre-cartilage and is distinctly forming outside the ear capsule. The chondrocranium is cartilaginous, the fenestra clearly demarcated, and the precartilaginous columella external to the membrana fenestræ (Fig. 52). The development at this stage closely parallels that of Necturus, as already described (p. 590). It seems to us safe, therefore, to add Cryptobranchus to Necturus and Ambystoma as a form showing the extra-otic appearance of the columella, and its early connection with the squamosum. It is likewise noteworthy that the columella in all the larvæ examined shows no cartilaginous connection with the otic capsule; and in the adult the fusion is very slender,—if indeed it exists. The source of the cells that form the columella could not be determined from the material at hand, although two embryos were examined. Neither operculum nor opercular muscle are developed.

It is interesting to note in concluding that Cryptobranchus is the only form in which there has been general agreement as to the connection of stilus with squamosum; not so, however, the relation to the facial nerve. Both Wiedersheim ('77) and Parker ('82b) described the relation correctly and subsequently altered their statements.

Megalobatrachus. From the description of Parker ('82b), Wiedersheim ('77), Osawa ('99), as from the dissection of a single specimen in connection with this study, suffice it to state that it shows essentially the same relations as Cryptobranchus. Osawa (as quoted) describes the stilus as joining the palatoquadrate. Parker states that the hyo-suspensorial cartilage (his epihyal) *articulates* with the palatoquadrate; in Cryptobranchus there is continuity.

Amphiuma.

In Amphiuma a single plate fills the fenestra vestibuli and is free except on its cephalic end where it is fused with the ear capsule which is cartilaginous. The very large stilus is articulated at its distal end with the squasmosum and continuous with a process of the palatoquadrate. Its relation to the facial nerve and blood vessels is typical. On both inner and outer surfaces of the fenestral plate there is a layer of bone (Fig. 18) which is continued for a short

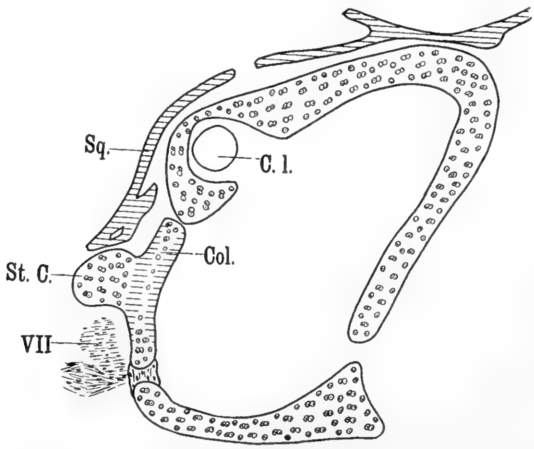


Fig. 18. Amphiuma means, adult. *C.l.*, canalis lateralis; *Col.*, columella; *Sq.*, os squamosum; *St.C.*, stilus columellæ; *VII.*, nervus facialis.

distance upon the base of the stilus. A distinct but not strong ligamentum hyo-columellare is present. Its diagrammatic representation is given in Pl. X, Fig. 71.

The fenestral plate becomes narrower in its caudal third and finally tapers to a point. In that portion of the ear capsule forming the caudal margin of the fenestra there is a pronounced prominence within which there is a continuation of the perilymphatic cavity of the ear (Fig. 20).

While complete developmental stages have not been studied, sections through the head of a larva soon after hatching, a transforming individual and an adult furnish some data regarding development. In the youngest larva the fenestral plate is relatively small

and free from the ear capsule all round. Although closely associated with, it appears to be wholly outside of, the fenestral membrane.

At transformation the columella is fused in front with the ear capsule and just fills the opening. Below and behind the columella a portion of the floor of the ear capsule becomes greatly thickened, especially in its lateral half, which is almost completely separated from the capsule (Fig. 19). In this stage there are a few muscle

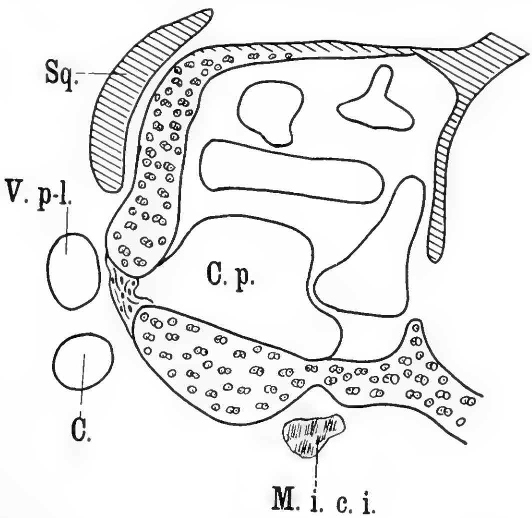


Fig. 19. Amphiuma means, transforming larva. *C.*, arteria carotis interna; *C.p.*, cavum perilymphaticum; *Sq.*, os squamosum; *V.p-l.*, vena petroso-lateralis; *M.i.c.i.*, a portion of the musculus intertransversarius capitis inferior.

fibers partly differentiated off from the M. intertransversarius capitis inferior which have a slight attachment to this portion of the ear capsule.

This portion of the floor of the ear capsule which was thickened and partly separated off in the transforming individual is in the adult very much thickened, forming the pronounced prominence which encloses an extension of the perilymphatic cavity as shown in Fig. 20. The M. intertransversarius capitis inferior comes into close relation with the prominence of the adult, but there is no differentiation of a distinct opercular muscle.

By comparing Fig. 11 and Fig. 31 (Pl. III) of Ambystoma and Fig. 50 (Pl. VII) of Triton it is evident that this portion of the otic floor corresponds in position to the operculum. It would seem as though at transformation a beginning of operculum formation was made which does not go through to completion.

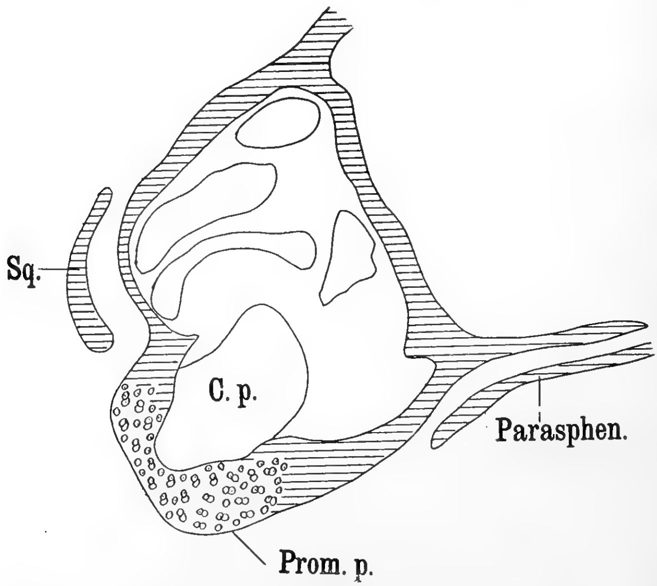


Fig. 20. Amphiuma means, adult. *C.p.*, cavum perilymphaticum; *Parasphen.*, os parasphenoideum; *Prom. p.*, prominentia perilymphatica. *Sq.*, os squamosum.

Siren.

The only material examined was an adult specimen 133 mm. long. The fenestra vestibuli is here, as in some other forms, filled by a single plate which is cartilaginous in our specimen although Cope ('88) states that it is bony in this species. It is elliptic in outline, facing outwards and downwards with a short stilus standing almost vertical to the plate and slight inclination being in a backward and downward direction. The connection of the columella with the suspensorium, if it ever existed, has disappeared. The relation of the stilus to the blood vessels and nerves in this region is typical.

The fenestral plate is fused throughout its entire cephalic margin with the ear capsule. This fusion is much greater in extent, relatively, than in either Typhlomolge or the Plethodontidæ.

The ceratohyal is much produced caudally, its falcate distal portion extending beyond the otic region. A short distance before the caudal extremity is reached there arises from the dorsal edge a broad and strong ligament extending forwards for insertion upon the fenestral plate (Pl. X, Fig. 72). The stilus projects directly into this just cephalad of which there arises another, the hyo-suspensorial ligament, which passes cephalad for insertion upon the palatoquadrate. These ligaments are considered by Cope as one, namely, a hyo-suspensorial ligament extending from the *quadrate* across the ear capsule to the exoccipital. Parker treats them as two separate and distinct ligaments. That portion extending to the quadrate he calls the hyo-suspensorial while to the columellar portion he applies the name suspensorio-stapedial. Functionally they are probably to be considered as distinct, but in serial sections they appear to be thickenings of the same sheet of fascia.

The short and free stilus projecting into the ligamentum hyo-columellare is more comprehensible in the light of the segmented condition of this structure in Typhlomolge. Thus in Siren all suspensorial communication of the fenestral plate with the exterior, such as is found in other forms, is cut off. But it will be noted that the ligamentum hyo-columellare in this form is strongly developed which together with its firm attachment to the fenestral plate may compensate for the absence of the usual skeletal connections. The relation of the stilus to the facial nerve and blood vessels is that found in the majority of forms.

Three figures (Pl. IX) illustrate the relations of the columella, stilus and hyo-columellar ligament. In Fig. 61 the short stilus is shown projecting out into the dense ligament, the diminutive plate joined to the margin of the fenestra by connective tissue. Fig. 62 is through the caudal end of the columella of the opposite side. In Fig. 63 the section is through the perilymphatic prominence, a caudo-lateral extension of the ear capsule. Slightly farther forwards the cartilage is hollowed out by a small recessus. The fenestra

and the caudal lip of the columella appear in the lateral wall of the prominence. In all essentials, save as noted, the sound-transmitting apparatus of the adult is like that of Amphiuma.

General.

In perusing the preceding portion of this paper, it will have become obvious that considerable confusion in the interpretation of the sound-transmitting apparatus in Amphibia,—particularly the Urodela,—has been caused by discrepant statements and conflicting descriptions of the conditions existing in the different forms. The differences that apparently existed concern principally (a) the relation to the suspensorium, (b) the relation of the facial nerve to the stilus columellæ, (c) the mode of development of the fenestral structures.

The Suspensorial Connection. Gaupp in his paper on the sound-transmitting apparatus of Vertebrates ('98) was led to the conclusion that there existed two types of connection between the suspensorium and the skeletal element filling the fenestra vestibuli,—one above and one below the facial nerve, and the same view has been taken by Drüner ('03). Gaupp later ('05) says: "Die oft citierte Angabe von Wiedersheim, dass der Nerv bei allen Urodelen ohne Ausnahme über diese Brücke hinweglaufe, ist irrig; vielmehr scheint der Verlauf der Nerven *ventral* von der fraglichen Brücke das häufigere zu sein. Letzteres findet sich bei Amphiuma (Hay 1890, auf Grund eines von Prof. Norris hergestellten Modelles kann ich diese Angabe bestätigen), Siredon (Hasse 1873, Parker 1877) und zahlreichen anderen Urodelen (Proteus, Desmognathus fusca, Spelerpes bilineatus, nach soeben veröffentlichten Untersuchungen von Kingsbury); der Verlauf des R. jugularis facialis *über* die erwähnte Verbindung findet sich bei Necturus (Huxley) und Proteus (Drüner, Kingsbury). Es sind somit 2 verschiedene suspensorio-columellare Brücken aus einander zu halten, eine über und eine unter dem Facialis (Gaupp 1899)."

For this divergence of statement, Huxley seems to have been primarily responsible, inasmuch as in Necturus he described a "Ligamentum suspensorio-stapediale" and stated that "the hyo-

mandibular branch of the seventh nerve (VII) passes above this ligament to its distribution, just as it passes above the *Columella auris* in the frog."

The real connection of the stilus with the special process of the squamosum[12] (*Processus columellaris*) he seems to have missed entirely. The reference to Huxley of a recognition of such a connection by Gaupp in the passage quoted above is incorrect. It should be remembered, however, that Huxley's observations were based upon minute dissections, in which a strand of fascia could easily be made to assume the appearance of a ligamentous structure, and the obvious passage of the hyomandibular division of the seventh nerve *over* the columella in the frog and the relation in mammals would incline one *a priori* to a similar interpretation in urodeles. Wiedersheim appears to have accepted his statement of relations, and without paying special attention to the point, gave as the universal condition in urodeles that the facial nerve passed above the suspensorial connection. Huxley's description doubtless influenced his friend, W. K. Parker,[13] who in several forms described the facial nerve as below the columella, yet sometimes located it above, or was vague in his descriptions.

One result of this study is to show that a "suspensorial connection" does indeed exist, in all save four of the salamanders examined (Diemictylus, Triton, Siren, Batracoseps) the nervus facialis being below and in front (see, however, p. 610). The connection is not primarily with the palatoquadrate, as has been heretofore believed, but with the bone, partly overlying the palatoquadrate and partly over the lateral semicircular canal of the ear, which we have regarded as *squamosum*. This primary connection of the columella through

[12]The correct relation was described subsequently by Cope ('88, '89), Wilder ('03), Drüner ('11), and Kingsbury ('03); the relation to the facial nerve, by the last two.

[13]Parker, '77 (p. 559), in a footnote says: "Professor Huxley pointed out this anomaly to me, showing me that this ligament cannot correspond to the "suspensorio-stapedial" ligament of *Menobranchus* (*op. cit.*, p. 192)." The reference is to the squamoso-columellar ligament in *Ambystoma*, which is, of course, *above* the facial nerve. See footnote on page 562 for Parker's description.

its stilus with the squamosum is now reported[14] for Necturus maculatus, Proteus, Typhlomolge, Typhlotriton, Spelerpes bislineatus and ruber, Gyrinophilus prophyriticus, Hemidactylium scutatum, Plethodon cinereus, Stereochilus marginatus, Autodax lugubris, Manculus quadridigitatus, Desmognathus fusca, Ambystoma punctatum, Chondrotus tenebrosus, Cryptobranchus allegheniensis, Megalobatrachus maximus.

In certain of the forms, however, at transformation,—before or after it,—the connection of the stilus tends to shift to the palatoquadrate, presumably in the change of the position and direction of the suspensorium to which Wiedersheim called attention ('77). In this displacement of parts as we interpret it, the squamosum, as in Ambystoma, comes to lie more on the dorsal surface of the palatoquadrate. In certain forms a special process of the palatoquadrate develops (e. g., Plethodon, Amphiuma) which may reach a relatively great length (Amphiuma). Hence we see how the more obvious connection has become accepted as the typical one, and the primary connection with the squamosum[15] is overlooked, even though occurring throughout life as the direct articulation in many of the forms. If the anatomical relation of parts is such that the palatoquadrate does not come into the proximity of the distal end of the stilus in the shifting, a connection with it is not established,—as in the case of Cryptobranchus (young adult) where the connection of the stilus moves from the caudal edge to the under side of the squamosum. Whether the stilus columellæ maintains the primary relation with the squamosum or becomes more closely joined to the palatoquadrate, any physiological value it may possess as a "suspensorial connection" remains unaffected, so closely is the lower

[14]Cope, '89, describes the correct condition in Necturus and Proteus (p. 22); of the remaining urodeles he says: "The stapes [columella] has no connection with the suspensorium in the adult except in the Cryptobranchidæ and Amphiumidae. It is connected with the suspensory cartilage, which is continuous with the quadrate cartilage in the latter families, and in the young of other Urodela" (p. 29).

[15]The development of the stilus columellæ has not been studied in Salamandra, nor in Amphiuma has the earliest development been yet worked out (see Kingsbury, '03, p. 325). The squamosal connection will doubtless be found in all salamanders possessing a well developed stilus.

end of the squamosum joined to the quadrate. From a morphological viewpoint, however, it is not a matter of indifferent significance. The connection of the columella with the squamosum and its formative cells is a primary one, while the direct articulation or junction with the palatoquadrate is only secondarily established. The significance of the relation becomes more impressive when the development of the columella is considered. In the forms in which the early development has been traced,—Necturus, Ambystoma, Cryptobranchus, Spelerpes, Plethodon,—before cartilage has appeared in the columella, when the squamosum is but a delicate scale of bone, a cord of cells proceeds from the proton of the columella to the cells underlying the forming bone. The developmental relations will be considered again in connection with the question of hyomandibular homology.

Columella and Operculum. A second complication exists in Amphibia due to the existence of two structures that are found fitting into the fenestra vestibuli, and the failure to recognize this fact has added another source of confusion in the elucidation of the relations in this group. It is the first of these two elements, distinguished by us as *Columella,* which possesses a suspensorial connection. The second, termed by us the *Operculum,* possesses no such relation. In its typical form as it exists in the adult Ambystoma, Diemictylus or Salamandra, the operculum is a spheroid filling in the definitive fenestra with whose lips it is connected by membrane only or possesses a slight cartilaginous connection at its caudal end. It may be massive (spheroidal) as in Salamandra, or more plate-like as in Triton. The cephalic end is included by the lip of the fenestra; the caudal end projects freely, covers the recessus perilymphaticus externally and affords attachment to the M. opercularis. In the families of the Plethodontidæ and Desmognathidæ the condition is characteristic and unique; the oval window has fitted into it a plate whose cephalic portion possesses a well developed stilus and whose caudal portion offers attachment to the M. opercularis and covers externally a recessus perilymphaticus; it seems therefore to *represent* both columella and operculum.

The statement of Gaupp ('05) in reviewing the Amphibian sound-transmitting apparatus (p. 605), that only by a stretch of the

imagination could an extra-otic origin be ascribed to the element filling the fenestra vestibuli in this group, was made in the light of the condition existing in Triton and the fragmentary developmental evidence already at hand, and without knowledge of the existence of two structures in urodeles. It applies, therefore, with full force to the development of the operculum. The columella, however, develops in quite a different manner, and here the evidence of an extra-otic origin is much stronger. Killian ('90) first described this structure as developing outside the ear capsule in Ambystoma, and Miss Platt ('97) in Necturus advanced the same view. Kingsbury subsequently examined the condition in larval and embryo Necturi and came to the same conclusion. In this paper we have shown evidence of an entirely similar mode of origin of the columella, outside the fenestral membrane, in Cryptobranchus, Spelerpes, and Plethedon, and confirmed Killian's observations on Ambystoma. Supplementary evidence of an extra-otic origin for the columella is to be found in the fact that from the first, before chondrification has begun, the columella is connected by a dense grouping of cells with the squamosal cells around the outer side of the vena petroso-lateralis.

With the growth of the fenestral plate of the columella it becomes associated or fused with the cephalic edge of the fenestra. This is not, however, a primary connection but a secondary one. Hence the statement that has been made (Winslow, Stöhr) that the columella grows out of the cephalic edge of the window is not correct. In the descriptive portion of this paper, the difficulty in determining just what occurs in the chondrification of the plate of the columella has been pointed out. Even though the columella grew through the incorporation of otic tissue, its origin from without the otic capsule would not be invalidated, which is supported by its relation before chondrification and its early and intimate connection with the subsquamosal cells.

The tendency of the columella to become fused with the cephalic cartilaginous margin of the fenestra is in some forms carried to an extreme, and results in a more or less complete incorporation of the structure into the ear capsule by extensive fusion with it. In the Ambystomidæ its boundary is still distinguishable by the charac-

teristic inner and outer bony plates, and by the stilus. Salamandra lacks the ossification, the incorporation appears more intimate but the stilus is persistent. In Triton and Diemictylus stilus and ossification are both lacking and incorporation into the edge of the fenestra is complete.

Attending the fusion of the columella, there is the formation of the operculum to inherit its position and function as a fenestral structure. Concerning its origin and development there is not, as in the case of the columella, opportunity for uncertainty. In Ambystoma, in which it appears late,—at the end of transformation,—it is cut out of the floor of the otic capsule medial to the fenestra. In Diemictylus and Triton, in which it appears early, "cutting out" must play a more secondary part, and marginal growth by chondrification of opercular tissue seems more important. It occupies in either event the same position and seems to be a detached portion of the otic capsule, although extensive cartilage formation in it may give it a thickness several times that of the otic capsule. It undergoes no ossification, remaining cartilaginous throughout life.[16] Its characteristic features, negative and positive, are the lack of connection with the skeleton outside the ear capsule, and the attachment of the M. opercularis.

The fusion of the columella causing a more or less extensive filling in of the cephalic portion of the fenestra vestibuli necessitates the recognition of primary, secondary and definitive fenestræ, of somewhat different boundary and location, as in the Anura (Gaupp, '93). The question of in how far the cephalo-caudal succession of fenestral elements or the (essentially) cephalo-caudal extension of the fenestra itself is an expression of the play of factors of relative growth of parts,—otic capsule, suspensorium, etc.,—is simply raised. It is hardly necessary to state that the primary character of the fenestra as an unchondrified portion of the otic capsule, as affirmed

[16]It is interesting to find Parker ('82a) commenting on this condition; on page 199 he says: "One more point of interest is to be noted here: the lowest Perennibranchs have their stapes thoroughly ossified (*i. e.*, the columella); the highest Caducibranchs, like the Batrachia, have it (*i. e.*, the operculum) soft; here in *Spelerpes* it ossifies early, and becomes a very perfect and elegant shutter to that small oval window."

by Stöhr and more recently by Fuchs ('07) as contrasted with an appearance secondarily, by dehiscence or absorption, is abundantly established. It must be understood, however, that this applies only to the primary fenestra, while in the formation of the secondary and definitive fenestræ, in some forms, at least, the extension is accomplished essentially as a "dehiscence." The developmental transformations undergone by the primary fenestra require a more detailed study in a number of salamanders before a full interpretation can be made.

In the Plethodontidæ and Desmognathidæ, where the single fenestral plate possesses some of the characters of operculum and columella combined, the development has been followed in *Spelerpes bislineatus.* There is no evidence of a fusion of two structures, the cartilaginous and bony plate which bears the stilus by growth gains the territory which in Ambystoma is occupied by the operculum and may therefore be regarded as representing that structure without embodying it.[17]

On plate X are set forth in schematic form the morphological relations of the two structures under consideration as they occur in typical forms. Examining the conditions in the Urodela, we find that they may, with the figures of this plate as illustrations, be divided into seven groups, the characters being compared in tabular form on page 607.

Under these seven groups the forms studied distribute themselves as follows: Group I; Necturus, Proteus (Typhlomolge). Group II; Larval Ambystoma and urodele larvæ in general, Cryptobranchus, Megalobatrachus, Amphiuma. Group III; Adult Ambystoma, Adult Chondrotus, probably other members of the Ambystomidæ. Group IV; Salamandra, and possibly some of the Ambystomidæ. Group V; Diemictylus, Triton, undoubtedly Salamandrina and other Pleurodelidæ. Group VI; Siren. Group VII; the Plethodontidæ (as examined),—Typhlotriton, Spelerpes, Gyrinophilus, Hemidactylium,

[17]The differences between the Ambystomidæ and the Plethodontidæ in respect to the presence and absence of an operculum, are paralleled in the Anura by the similar differences in the Frog (as an example of the type) and Pipa. Gaupp's very interesting discussion of the interpretation in the last case ('98, p. 1065) presents the problem for the first case as well.

Plethodon, Stereochilus, Autodax, Manculus (Batracoseps). The Desmognathidæ (Desmognathus fusca.)

Two forms are exceptional; *Batracoseps,* which otherwise falls under group VII, possesses a vestigial stilus and therefore lacks the connection with the suspensorium, usually well developed in the Plethodontidæ. The interesting form *Typhlomolge* is plethodontid in the character of the fenestral plate, but possesses a fragmented stilus. The interesting course of the R. jug. VII over the stilus, and the absence of the M. opercularis, however, places it in Group I.

Table to Show the Seven Types of "Columella auris" in Urodeles.

Group		Columella					Operculum	M. operc.
	Fig.	Fenestral plate	Fusion with fenestral margin	Stilus	Connection of stilus with	Relation of R. jug. VII to St.		
I.	68 69	present	none or slight	present	squamosum	above	absent	absent
II.	70 71	present	none or slight	present	squamosum or Sq. and Pal.-Quad.	below	absent	absent
III.	64	present	extensive	present	squamosum and palato-quadrate	below	present	present
IV.	65	present, cartilage	complete	present	palato-quadrate	below	present	present
V.	66	present	complete	absent	absent		present	present
VI.	72	present	moderate	present?	ceratohyal		absent	absent
VII.	67	prseent	slight	present	squamosum, quadrate, or palato-quadrate or all	below	not separately developed	present

Briefly classified from the point of view of the existence of two fenestral structures, *Columella* and *Operculum,* these forms may be divided into the following groups: A, groups I and II; the columella present, free, connected with the suspensorium, no operculum or opercular muscle. B, groups III and IV; the columella present, fused with the otic capsule, connected with the suspensorium, operculum and opercular muscle present. C, group V; columella vestigial, fused with the ear capsule, no connection with the suspensor-

ium. Operculum and opercular muscle present. D, group VII; columella present, joined to otic capsule, connected with the suspensorium; the opercular muscle present, the operculum not developed as such. Up through the groups I to V, evidence of indubitable value is presented of the incorporation and loss of an element (columella) with an attendant loss of its primary connection, a substitute making its appearance (the operculum). The m. opercularis, absent in groups I, II and VI, present in the others, appears to deserve the name given it by Gaupp ('93), since in the Urodela it possesses characteristic attachments and is not a portion of another muscle (Levator anguli scapulæ). When an operculum is developed, an opercular muscle is present, while its insertion in group VII is suggestive, as is also equally, its absence in groups I and II. From the examination of larvæ, it should be stated, however, that it appears to be absent in all urodeles in the larval state. It will be referred to subsequently in connection with the problem of the function of the urodelan "sound-transmitting" apparatus.

Comparison with Anura. In the first contribution a comparison was made of the conditions found in Ambystoma to the "columella auris" of the frog, and further investigation has strengthened us in the acceptance of the homologies then advanced,—the columella with the plectrum (pars interna plectri), the operculum being homologized in the two groups. The resemblances and differences between the two forms may perhaps best be presented in tabular form, in parallel columns the resemblance being presented first, enumeration of differences following. The relations in the frog are taken from Gaupp ('93, '05).

The first of the differences (No. 7) is of no marked significance, since in other Anura (e. g., Dactylethra, Parker, Gaupp '98) the plectrum may overlap the operculum. The next two contrasts partake more of the nature of resemblances than of differences, hence items 10 and 11 are the only points of difference needing comment here, a detailed discussion not being intended, since they are doubtless the ones that will be regarded as the most weighty.

As to the tenth item, it is only necessary to recall the numerous instances of precocious and postponed development of homologous

	Frog.	Ambystoma.
1.	Two independent elements (*a*) Pars interna plectri (*b*) Operculum.	Two independent elements (*a*) Columella (*b*) Operculum.
2.	The plectrum develops in the cephalic, the operculum in the caudal portion of the secondary fenestra.	The columella develops in the cephalic, the operculum in the caudal portion of the secondary fenestra.
3.	The operculum chondrifies in the opercular tissue and becomes joined to the cartilaginous margin of the fenestra on its dorso-caudal side; becomes completely separated.	The operculum is cut out of the ear capsule, remaining connected longest at its caudal end; becomes completely separated.
4.	The plectrum chondrifies as an independent center out into the dense tissue connecting it to the palatoquadrate.	The columella chondrifies as an independent center in dense tissue connecting it with the squamosum (and palatoquadrate).
5.	The plectrum becomes connected with the ventral (cephalo-ventral) edge of the fenestra.	The columella becomes fused with the cephalic (cephalo-ventral) edge of the fenestra.
6.	The M. opercularis inserts upon the Operculum.	The M. opercularis inserts upon the Operculum.
7.	The cephalic end of the operculum *overlaps* the fenestral plate (pseudoperculum) of the plectrum.	The cephalic end of the operculum *underlies* the columella.
8.	The cephalic portion of the secondary fenestra closes, *excluding* the pseudoperculum.	The cephalic portion of the secondary fenestra closes, *including* (incorporating) the columella (fenestral plate).
9.	A cephalic extension of the cavum perilymphaticum is beneath the pseudoperculum (Ductus fenestræ).	A caudal extension of the cavum perilymphaticum is beneath the operculum (Recessus perilymphaticus).
10.	The operculum develops first; the plectrum at transformation.	The columella develops first; the operculum at transformation.
11.	The hyomandibular nerve passes *above* the plectrum.	The hyomandibular nerve (VIIth) is *below* the stilus columellæ.

structures in different forms (Heterochronia) to appreciate that the relative reversal of sequence in frog and salamander is not intrinsically important. Among the Urodela themselves we have an instance of partial reversal, the operculum appearing early in Triton (about 18 mm.) and late in Ambystoma (at transformation).

The opposite relation of the nervus facialis to the plectrum and stilus columellæ, is perhaps a more serious difference, since it is just this criterion of relative position of sound-transmitting apparatus and nerve that has been considered of decisive moment in the determination of homology. As has been stated above, Huxley introduced confusion into the interpretation in Amphibia by faulty recognition of indifferent connective tissue as the equivalent of the frog's plectrum, hence the course of the facial nerve came to be interpreted as *over* the suspensorial-columellar ligament or stilus, whereas in all forms examined just the opposite relation holds,—the facial nerve is *below* (cephalad or ventrad of) the columella, with three exceptions,—Necturus, Proteus, and Typhlomolge,—and in these cases the *jugular branch alone passes above while the rami mandibulares, internus and externus, are below* and in front of the columella.

If the different relation of the facial nerve to the columella in Urodela and Anura is prohibitive of the plectrum-columella homology, there is necessary the recognition of three morphologically distinct squamoso-columellar connections in tailed amphibia, (1) in the Proteida, (2) in Typhlomolge, (3) the remaining urodeles. This, it is felt, is contrary to the ontogenetic evidence. There is involved in this point the broader question of the value of the relation of nerve to skeletal structure as a criterion of homology. The real value of such a test of homology has already been questioned by one of us (Kingsbury, '03, p. 333) and a few instances given of variation in the relation of nerve, ligament and muscle,—instances which could, of course, be easily multipled many fold; for example, in the relation of the hyomandibular bone or cartilage to the hyomandibular nerve, in fishes, etc. It was not deemed desirable to introduce here further consideration of such evidence, as it would involve work along broader comparative lines than was desirable at this time. Adherence is given to the view entertained in the first contribution,—

that the columella and its suspensorial connection is homologous throughout the urodele group, and no valid reason is known why the homology should not be extended to include the Anura as well. Inasmuch as a study of the relations and development in that group has not been made, the homology of columella and plectrum is not presented with the emphasis of personal investigation.

Ligaments. The only muscle that is in the region of the head involved in this study is the *Musculus cephalo-dorso-mandibularis* (*M. depressor mandibuli*). It has its origin largely, and in some of the forms entirely, from the squamosum, and inserts by means of a longer or shorter tendon upon the retro-articular process of the os articulare. Beneath the muscle, between it and the ear capsule, palatoquadrate, hyoid and mandible, is the space filled in with connective tissue containing the nerves and blood-vessels. The tissue next the muscle is denser and comes into intimate relation with the squamosum, palatoquadrate, stilus columellæ, ceratohyal, and mandible. (For illustration, see Figs. 43, 51, 55, 61.)

This submuscular fascia appears to be the foundation, out of which, by thickening of different portions three ligamentous structures may be formed:—(a) Ligamentum hyo-suspensoriale (palato-quadrate), (b) Ligamentum hyo-mandibulare, (c) Ligamentum hyo-columellare. These may all be present in the same species, weakly or strongly developed. Of these the last two have long been recognized (Huxley '74) and are in general well described; the hyo-columellar ligament, however, has not been as adequately considered and is of more interest here because of its connection with the columella. It is best developed in Siren (Fig. 61, 62, 63, Pl. IX), but its presence has been mentioned in the first part of this paper in Amphiuma, Cryptobranchus, Gyrinophilus, Spelerpes, Desmognathus; and seems to be more or less well developed in the Plethodontidæ generally. It will be referred to subsequently in connection with the discussion of the function of the urodelan apparatus. It was not recognized in Ambystoma, Triton, Diemictylus or Salamandra.

A ligamentum palatoquadrato-columellare corresponding to Huxley's suspensorio-stapedial ligament described by him in Necturus it noticeably absent in that form as well as in other urodeles.

Beneath the facial nerve, between columella or operculum and palatoquadrate, is only loose connective tissue, denser in some forms it is true,—especially Siren which possesses well developed hyo-columellar and hyo-suspensorial ligaments giving a less direct suspensorio-columellar bridge of dense connective tissue. It does not seem in any form to possess relations of functional or morphological importance as a "suspensorial" connection of the "sound-transmitting" apparatus.

While the presence or absence and relative development of these ligaments appears to be an expression of the mechanical needs, requirements of support in the nature of a physiological adaptation and therefore secondary phylogenetic importance, their existence may possibly have a deeper significance; the ligamentum hyo-columellare, for example, indicating a primary relationship of columella to the hyoid arch.

The junction of the ceratohyal with the palatoquadrate is quite constant. It is in relation to the hyo-quadrate ligament that the processus hyoideus palatoquadrati is developed in so many forms (see Wiedersheim '77, p. 533).

The diagram reproduced as Fig. 21 illustrates the relations of these three ligaments.

The Hyomandibular-Symplectic Homology. It may be safely stated that from the phylogenetic side the hyomandibular homology of the Amphibian sound-transmitting apparatus is at present generally accepted, as may be seen from the statements in the works of Wiedersheim and Gaupp which were cited (p. 552) as expressing the more recent interpretation. In the earlier form of the theory, however, only a portion was given a hyoid or hyomandibular homology; Reichert, Huxley, Parker, and others regarding the operculum as purely of otic origin.

This hyomandibular homology has been reiterated despite a dearth of evidence from the ontogenetic side. In the Anura, it is true, the embryological evidence has been furnished especially by the monograph of Gaupp, and it has seemed to support the partial homology of the older workers,—if supporting it at all. No direct connection has been found with the ceratohyal in development. In

the Urodela the condition is less satisfactory. Parker, Wiedersheim and Stöhr had emphatically declared the origin of the operculum from the ear capsule and the absence of any association in development with the hyoid arch.

More recently in a paper before the Anatomische Gesellschaft Fuchs ('07) rejected completely the hyomandibular homology[18] of the amphibian structures, basing his conclusions upon the otic origin of the operculum in Salamandra (See p. 568). The presence of a stilus was regarded by him as secondary, the operculum without stilus representing the primitive condition. In the discussion, the homology with the hyomandibulare was upheld by several. Gaupp at that time ('07, pp. 31 and 32) stated his conception of the primitive "Columella auris" as consisting of an operculum and a stilus, the latter articulated with the palatoquadrate. Since the hyomandibulare in fishes is, in many forms, between the palatoquadrate and the otic capsule, the connection of the stilus with the palatoquadrate strongly suggests the homology. He admitted the lack of ontogenetic evidence, but pointed out the difficulty attending the delimitation of what might be hyal blastema from the periotic blastema. Since the upper end of the hyoid arch closely adjoined the otic capsule, blastema of the hyoid arch, it is easily conceivable, might be early incorporated with the periotic, and the operculum might develop apparently as part of the ear capsule and nevertheless be of hyoid origin. In his rejoinder Fuchs (pp. 33, 34) affirmed his belief that the operculum without stilus was the more primitive; that the fenestra arose as a result of the action of a "biological factor" within the ear itself upon the assumption of terrestrial life; that the "rubbing" of the hyomandibulare could in no wise have caused the appearance of the fenestra vestibuli, since in the numerous fish forms

[16]Fuchs ('07, p. 24): "Ich erachte das Operculum bezw. Operculum + Stilus der Urodelen für homolog dem Otostapes der Reptilien und beide für homolog dem Stapes der Säugetiere. Alle drei halte ich ontogenetisch für Abkömmlinge der Gehörkapsel. Auch in phylogenetischer Hinsicht leite ich auf dieselbe zurück und bin ferner der Ansicht, dass sie nicht mit der Hyomandibula der Fische zu vergleichen sind." "Für das Operculum, eventuell Operculum + Stilus, der Urodelen wird dem ja wohl niemand mehr widersprechen. . . ."

in which it does come in contact with the periotic cartilage, no trace of a thinning or fenestration results. Commenting on the position of the hyomandibulare between palatoquadrate and the otic capsule, he expressed the conviction that we must look for the ancestors of terrestrial vertebrates in the low elasmobranchs (Notidanidæ and Pro-Selachia) in which the hyomandibulare has no part in the suspension of the jaw, but affords the hyoid arch an independent articulation with the skull.

Since our results bear directly on the problem and from some points of view, at least, seem to strengthen the homology of the amphibian columella auris with the hyomandibulare, illuminating some of the obscure points of development and relation, it seems desirable to consider it briefly from the following view-points: (a) the extra-otic origin of the columella, (b) the relation to the palatoquadrate, (c) the connection with the squamosum, (d) the relation to the facial nerve, (e) the relation of the columella auris to the ceratohyal.

As Fürbringer pointed out in the discussion of the paper of Fuchs, Salamandra is highly specialized and not a form upon which to base conclusions of general applicability. This has been markedly illustrated in this very problem. For the present, the operculum (our use) may be regarded as a part of the otic capsule both in this and the other forms that possess this structure. Fuchs, however, dealt with but half the problem, as has been shown (p. 568). By other workers, in other forms evidence has been given of an extra-otic origin of a part, at least, of the fenestral structures:—In Necturus by Miss Platt and by Kingsbury (not published); in Ambystoma by Killian and by ourselves in this paper; in Cryptobranchus, Spelerpes and Plethodon, in this paper. In these salamanders, and doubtless in all forms possessing a well developed columella (our use), the first appearance of the sound-transmitting apparatus is as a group of cells outside the otic capsule. Of this the evidence seems quite strong; it is only necessary to refer once more to the figures illustrating the condition (Pl. IV, Fig. 39; Pl. VII, Figs. 52 and 53; Text Figs. 6 and 7). In the growth of this blastema in close association with the otic capsule, the chondrification as stilus and fenestral plate, there is introduced the difficulty mentioned by Gaupp,

namely, that of distinguishing cells of extra-otic origin from the periotic blastema. It has, in fact, been so far impossible in Ambystoma and Spelerpes to determine whether or not the fenestral plate of the columella embodied a periotic element. It may be said, however, that in its chondrification and growth the stilus is an integral part of the columella as a whole and not an element from outside secondarily fused with the plate,—representing, therefore, a structure which could by itself be homologized with the hyomandibulare, as was done by Parker.

The articulation of the stilus columellæ with the palatoquadrate, which had been mentioned, as quoted above, by Gaupp (cf. Wiedersheim '06, Gaupp '05) as the significant relation for the hyomandibular homology, has been shown to be a connection secondarily established during growth and development apparently due to a shifting of the connection from the squamosum to the palatoquadrate, in many forms a longer or shorter process of that cartilage developing with which it is articulated or fused. If this connection is in the nature of a secondary adaptation,—and this interpretation is the more probable,—its phylogenetic significance becomes doubtful. There is, however, an association of the two structures of a primary ontogenetic character and suggestive of profound significance. It is to be recalled that in those forms in which the early development of the columella has been traced, the cell mass constituting the proton of the columella is directly connected with the cells between squamosum, the otic process of the palatoquadrate and the otic capsule external to the lateral semicircular canal. If a connection with the palatoquadrate is to be considered of value in determining the hyomandibular homology, it would seem to us, therefore, to be expected that in the displacement of the hyomandibulare from the suspensorium, it would be that portion of the upper end of the palatoquadrate which comes to articulate with the otic capsule with which the homolog of the hyomandibulare would be joined rather than a point farther down. If, on the other hand, as maintained by Fuchs, the ancestry of the Amphibia is to be sought far back among the primitive elasmobranchs, it is in this region,—over the lateral semicircular canal caudad of the otic articulation of the palatoquadrate,—that the connection of its vestigium might be sought.

One of the characteristics of the urodele stilus is its early connection with the ventral edge of the squamosum, and from the first it has appealed to us as of profound morphological significance. Its final interpretation, however, is not yet possible, and must await the establishment of many facts of relation and development in the domain of comparative morphology. Although from the development of this bone over the lateral semicircular canal in urodeles (Kingsbury '03; Thyng '06) it is regarded by us as a squamosum, other considerations led Gaupp to bestow upon it the indifferent name of *Paraquadratum*. Granting that it is a squamosum, its development requires further study, and its value in urodeles,—as Dermo-, Auto- or Amphisquamosum has to be considered. As an Autosquamosum deriving its cells (primarily?) from the periotic blastema, it may be pointed out that it has precisely the position and relation to the proton of the columella that would be required of it if the hyomandibular homology of the latter is accepted, since in fishes it seems to afford a portion of the articular surface for that bone. Nor do two considerations that immediately occur appear necessarily antagonistic to this view; the first, that in the event of the acceptance of the elasmobranch origin of the Amphibia, no squamosum (as such) existed in the ancestral forms; the second, that the larval connection of the columella with the squamosum is but a step in the shifting of the suspensorium (palatoquadrate) during growth, which in its second phase again transfers the connection to the palatoquadratum or the os quadratum.

The absence of any connection between the columella and the ceratohyal has possibly been most commonly regarded as the greatest defect in the ontogenetic evidence for the hyomandibular homology. Recognition of the existence of a columellar proton outside the otic capsule, its relations to palatoquadrate, otic capsule, and facial nerve, in comparison with the requirement of a first segment of the hyoid arch, renders less important evidence of a connection of ceratohyal and columella. Yet the evidence of an embryological relation of the two structures seems stronger than is usually recognized. Miss Platt ('97), when describing the extra-otic origin of the columella (her operculum) considered that its lack of connection with the

ceratohyal did not "demonstrate that the cartilage in question may not be a rudimentary element of the hyoid arch, since each element of the cartilaginous arches arises from an independent center of chondrification and secondary fusions of cartilaginous elements do not necessarily show original association."

The cord of cells which extends to the under surface of the squamosum and which must be regarded as a part of the columella blastema, is joined by an extension from the perichondral cells of the ceratohyal, so that, at an early stage (19 mm.) the two structures are in fact connected (Kingsbury '03, p. 318, and Fig. 2*a*). In Ambystoma there is a line of cells from the ceratohyal to the columella in the 13-14 mm. specimen. In Plethodon (Text Fig. 6) the juxtaposition is still closer and a cellular continuity exists. In Cryptobranchus the ceratohyal is not joined to the columella by cells in the just hatched larva; just what the condition is at an earlier stage we cannot say from lack of material at a suitable stage.

This early connection by cells does not seem a chance association, but rather to indicate that columella and ceratohyal chondrify out of a common blastema. The Ligamentum hyo-columellare previously described as existing in several forms seems to be a secondary development, though it may express a primitive relationship. The diagram introduced to illustrate the ligaments (Fig. 21) may also serve to indicate the skeletal connections and relations of the columella as a probable hyomandibulare.

The position of the columella above and behind the nervus facialis satisfies the requirements of the hyomandibular homology. It is but necessary to recall its position in elasmobranchs and Polypterus (Ruge '96, Pollard '92) to recognize this fact. No detailed study has been made of the relation of this nerve in fishes, however; nor has the significance of the rather constant position of the columella between the vena petroso-lateralis and the arteria carotis interna been investigated.

The connections and development of the columella do, we believe, strengthen its homology with the Hyomandibulare of fishes, in support of which so much has been written.

Function of the "Sound-Transmitting" Apparatus.

In order to determine the precise function of the so-called sound-transmitting apparatus in Urodeles a series of experiments upon both larvæ and adults is necessary. The skeletal connections of the fenestral elements in the larva, the changes taking place upon the assumption of the terrestrial life and the correlation of structure and habit in certain adults furnishes, however, some basis for judging the function of these structures.

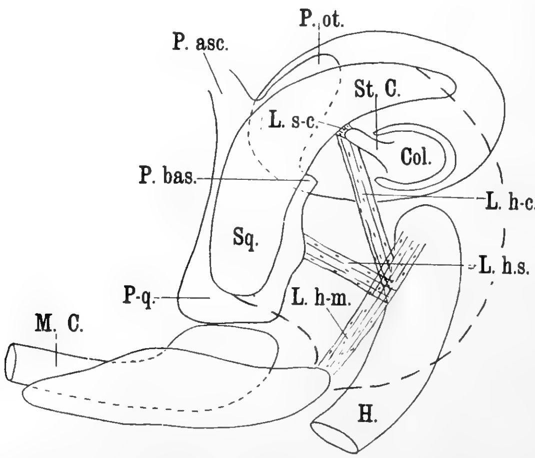


Fig. 21. Schema to illustrate the relation of the ligaments found in this region. *Col.*, columella; *H.*, ceratohyale; *L.h-c.*, ligamentum hyo-columellare; *L.h-m.*, ligamentum hyo-mandibulare; *L.h-s.*, ligamentum hyo-suspensoriale; *L.s-c.*, ligamentum squamoso-columellare; *M.C.*, Meckel's cartilage; *P.asc.*, processus ascendens palatoquadrati; *P. bas.*, processus basalis palatoquadrati; *P.ot.*, processus oticus palatoquadrati; *Pq.*, palatoquadratum; *St.C.*, stilus columellæ; *Sq.*, os squamosum.

It is perhaps not assuming too much to believe that the sound-transmitting apparatus serves as an organ of equilibration and the detection of vibrations of low frequency when such come to the animal through a dense medium such as earth or water. The nature and structure of the apparatus in urodeles seem to preclude the belief that vibrations of any frequency whatsoever can be detected from a medium as rare as the air. There is, however, nothing in the nature of the apparatus to interfere with the view that vibrations

of high frequency are not detected if transmitted to the animal through a dense medium. Experimentation along this line may prove very interesting and valuable.

During the larval period, and in aquatic or partly aquatic forms during adult life, the connection of the fenestral plate is with the suspensorium. Thus it would appear that jars or vibrations are transmitted from the objects, upon which they are resting, to the floor of the mouth, after which the course is through the suspensorium to the columella and thence to the inner ear, as has been

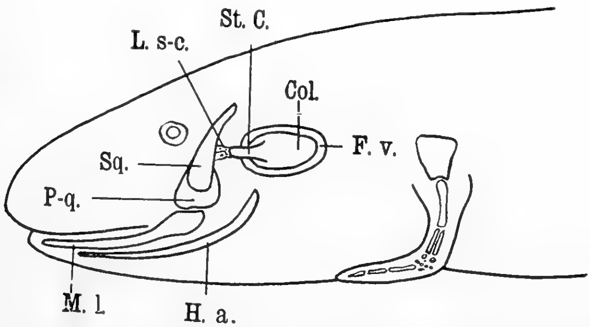


Fig. 21a. Schema to illustrate the possible method of communication between the inner ear and the exterior for larvæ and thoroughly aquatic adult forms. *Col.*, Columella; *F.v.*, fenestra vestibuli; *H.a.*, hyoid arch; *L.s-c.*, ligamentum squamoso-columellare; *M.l.*, skeleton of the lower jaw; *Pq.*, palotoquadratum; *Sp.*, os squamosum; *St.C.*, stilus columellæ.

suggested by Gaupp ('05, '07). The possibility of such a functional mechanism is illustrated in Fig. 21*a*.

At transformation when terrestrial life is assumed, in certain forms, a different type of communication with the exterior is formed. The columella becomes fused with the ear capsule while a free plate, the operculum, is formed behind and below as in Ambystoma or the caudal portion of the fenestral plate remains free as in the Plethodontidæ and some others. This plate is placed in communication with the exterior by the M. opercularis, connecting it with the shoulder girdle. Thus jars and vibrations may be transmitted through the arm to the M. opercularis, thence to the operculum and finally to the inner ear, as shown in Fig. 21 *b*.

These connections appear to be an adaptation to the habits and environment of the animal. During larval life and in such forms as Necturus and Cryptobranchus the body is either supported by water, or rests full length upon the bottom or some submerged object, the arms and legs playing a small part, if any, in its support. The primitive connections of the columella here persist and function as the transmission line between the exterior and the inner ear. Some evidence for this view is found in certain Anurans. In Pelobates there is no tympanic cavity, and the eustachian tube is much reduced. The fenestral plate in this form is connected by strong ligament

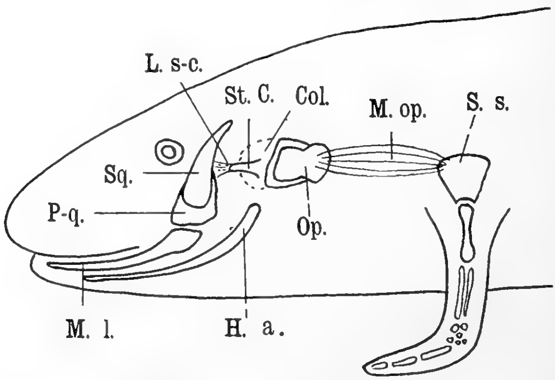


FIG. 21b. Same as Fig. 21a, for terrestrial forms. *Col.*, columella; *H.a.*, hyoid arch; *L.s-c.*, ligamentum squamoso-columellare; *M.op.*, musculus opercularis; *M.l.*, skeleton of lower jaw; *Op.*, operculum; *Pq.*, palatoquadratum; *Sq.*, os squamosum; *S.s.*, suprascapula; *St.C.*, stilus columellae.

with the squamosum. Similar conditions obtain in Bombinator, excepting that the fenestral plate is connected with the hyoid. In either case the inner ear is placed in communication with the exterior through the floor of the mouth. It is interesting to note in this connection that Pelobates is a thoroughly burrowing form while Bombinator is just as thoroughly an aquatic one.

In the urodele Siren, where the usual skeletal connections of the fenestral plate are wanting, they seem to be compensated for by the attachment of the stilus columellæ to the hyoid by means of the strong hyo-columellar ligament.

When terrestrial life is assumed the body is more or less supported

by the limbs, thereby raising the floor of the mouth above the ground. Thus communication of the internal ear with the exterior through the suspensorium and floor of the mouth is lost. By the detachment of a portion of the M. intertransversarius capitis inferior which becomes the M. opecularis, the operculum is connected with the shoulder girdle and communication of the inner ear with the exterior is again established,—through the arm and shoulder girdle.

Summary.

While to a certain extent (hyomandibular homology) comparison has been made with fishes, comparisons between the amphibian auditory apparatus and those of reptiles and mammals have not been attempted. The terms "stapes" and "stapedial" have been purposely avoided since it has seemed to us by no means conclusively shown that the amphibian columella is simply the homolog of the first segment of the mammalian chain of bones. Although we have fully appreciated that this investigation is but a part of the larger problem as was indicated at the beginning of the paper, the main aim has been the determination of *facts* for a large number of tailed amphibians, realizing that weaknesses in broad generalizations are in most instances due to drawing conclusions from insufficient data; for illustration of which it is unnecessary to go outside the limited field of investigation covered by this paper. The amphibian group presents within itself many problems of both local importance and general bearing that require further investigation, and it was this narrower field that particularly engaged our attention. Some of the general aspects of the problem we hope to deal with subsequently.

The main results were given in the first portion of the paper; they are restated below in somewhat extended form.

1. Several (4 to 7) different types of "columella auris" are found in urodeles.

2. There are two morphologically distinct fenestral structures found in the group: Columella and Operculum.

3. The Columella possesses a stilus, tends to fuse with the cephalic portion of the fenestral margin, and in ontogeny seems to come from outside the otic capsule.

4. The Operculum develops out of the otic capsule of which it appears to be primarily a part. It possesses no stilus but gives attachment to a muscle (M. opercularis). Its morphological position relative to the Columella is caudal and medial.

5. A Columella only is present in Necturus, Proteus, Cryptobranchus, Amphiuma, Siren.

6. In Ambystoma and Chondrotus (Ambystomidæ) a Columella is present in the larva but it becomes fused with the otic capsule at transformation, and an Operculum is then developed.

7. A vestigial and fused Columella is found in Salamandra, Triton and Diemictylus (Salamandridæ, Pleurodelidæ, Cope).

8. An Operculum is found (in the adult) in the Ambystomidæ, Salamandridæ, Pleurodelidæ.

9. The Plethodontidæ and Desmognathidæ possess a single fenestral structure bearing a stilus but also giving attachment of the opercular muscle.

10. Typhlomolge possesses a fenestral plate of plethodontid character. It has a fragmented stilus and lacks the M. opercularis.

11. The Musculus opercularis is absent in (a) Necturus, Proteus, Cryptobranchus, Amphiuma, Siren, Typhlomolge; (b) in larvæ generally. It is present in the adult Ambystomidæ, Salamandridæ, Pleurodelidæ, Plethodontidæ, and Desmognathidæ.

12. The Stilus columellæ is distally joined to the Squamosum, Palatoquadratum, Quadratum, singly or in combination. In Necturus, Proteus, Cryptobranchus, Typhlomolge and larvæ generally (Ambystomidæ, Plethodontidæ, Desmognathidæ) it is directly connected with the squamosum.

13. During growth or at transformation the connection tends to shift, usually to the Palatoquadratum.

14. The stilus is fragmented in Typhlomolge, vestigial in Batracoseps and Siren, absent in Triton and Diemictylus. In Salamandra its distal end is fused with the Palatoquadratum.

15. In development the proton of the Columella appears to be outside the otic capsule in those forms in which its development has been traced (Necturus, Ambystoma, Spelerpes, Plethodon, Cryptobranchus).

16. The proton of the Columella is connected by a distinct strand of cells with a group of cells between Squamosum, Processus oticus palatoquadrati and the Prominentia semicircularis lateralis of the otic capsule.

17. The facial nerve is entirely below the Columella (stilus columellæ) in all forms except Necturus, Proteus, Typhlomolge; in these, one ramus (R. jugularis) passes above the stilus.

18. The relation of the stilus to the blood vessels of the otic region appears quite characteristic; i. e., below the vein and above the artery.

19. A Ligamentum hyo-columellare is present in Siren, Amphiuma, Cryptobranchus, Desmognathus and many Plethodontidæ.

20. The hyomandibular homology is favorably discussed.

21. The homology of the Columella of Urodela and the Pars interna plectri of Anura is accepted.

22. The Operculum is regarded as a secondary development in those forms which possess it.

23. From the standpoint of functional adaptation, there seem to be in the Urodela three types of communication of the internal ear with the exterior: (a) through the floor of the mouth, mandible, suspensorium and columella; (b) through the floor of the mouth, the hyoid arch and columella; (c) through the manus, pectoral girdle, M. opercularis and operculum.

24. Type (a) is the more usual and is found in typically aquatic forms. Type (b) is best represented in Siren. Type (c) occurs in Salamandra, Triton and Diemictylus. A combination of (a), (b), and (c) is found in the Plethodontidæ and Desmognathidæ.

25. There appears a close correlation of the type present and the habits of the form (aquatic, semi-aquatic, terrestrial, burrowing, etc.).

Cornell University,
Ithaca, N. Y., June 22, 1909.

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ABBREVIATIONS.

A. General Skeleton.

Col., columella.
H., ceratohyale.
H.a., Hyoid arch.
M.C., Meckel's cartilage.
M.l., Skeleton of lower jaw.
Op., Operculum.
Parasphen., os parasphenoideum.
P.asc., Processus ascendens palatoquadrati.
P.bas., processus basalis palatoquadrati.
P.ot., processus oticus palatoquadrati.
P.h-p., processus hyoideus palatoquadrati.
P-q., palatoquadratum.
Pt., os pterygoideum.
Q., os quadratum.
S.s., Suprascapula.
St.C., stilus columellae.
Sq., os squamosum.

B. Ear Capsule.

C.l., Canalis semicircularis lateralis.
C.p., cavum perilymphaticum.
Cr.s., crista semicircularis.
D.p., ductus perilymphaticus.
"F"., secondary fenestra vestibuli.
F.p., fenestral plate.
F.v., fenestra vestibuli.
L., lagena.
Prom. l., prominentia semicircularis lateralis.
Prom.p., prominentia perilymphatica.
R.p., recessus perilymphaticus.

C. Ligaments.

L.h-c., ligamentum hyo-columellare.
L.h-m., ligamentum hyo-mandibulare.
L.h-s., ligamentum hyo-suspensoriale.
L.s-c., ligamentum squamoso-columellare.

D. Nerves.

VII, nervus facialis (VII).
R.h.VII, ramus hyomandibularis VII.
R.c., ramus communicans (IX-VII).
R.j.VII, ramus jugularis VII.
R.m.e.VII, ramus mandibularis externus VII.
R.m.i.VII, ramus mandibularis internus VII.

E. Muscles.

M., musculus cephalo-dorso-mandibularis.
M.op., musculus opercularis.
M.i.c.i., musculus intertransversarius capitis inferior.

F. Blood Vessels.

C., arteria carotis interna.
V.p.-l., vena petroso-lateralis.

PLATE I.

Models of the ear region of the skull of Ambystoma punctatum, drawn from the side and slightly from the caudal and ventral aspects.

Fig. 22. Ambystoma punctatum, mature larva about 45 mm. in length, beginning transformation, gills about one-half absorbed.

Fig. 23. Ambystoma punctatum, about 42 mm. in length, transformation period, gills mere stumps. In comparison with Fig. 22, there is shown the partially formed operculum with the opercular muscle.

Fig. 24. Ambystoma punctatum, young adult, about 52 mm. in length. The columella is now nearly completely fused. Remnants of the secondary fenestra are marked "F." Operculum and opercular muscle.

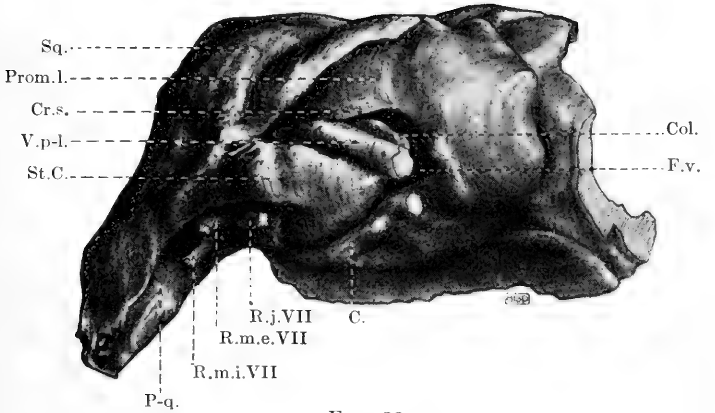

FIG. 22.

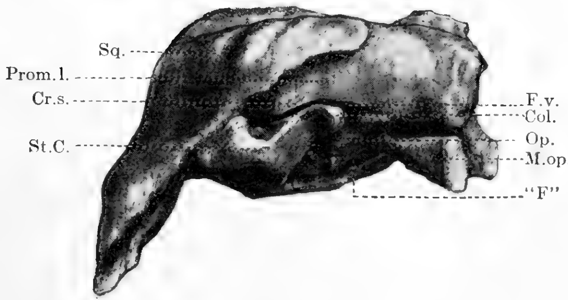

FIG. 23.

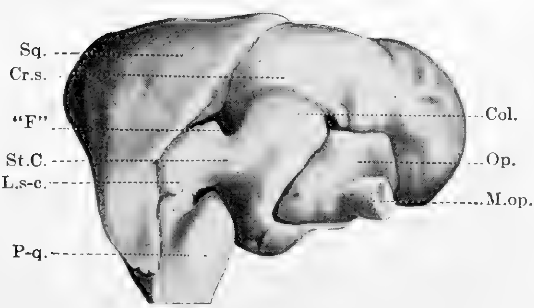

FIG. 24.

Plate II.

Drawings of models of the ear region of the skull.

Fig. 25. Ambystoma punctatum, adult. A portion of the ear region of the skull showing the completely fused columella and the fully formed operculum with its attached muscle.

Fig. 26. Salamandra maculosa, adult. The region and aspect as in Fig. 25 with which it may be directly compared. The boundary between muscle and operculum is indicated by the broken line.

Fig. 27. Triton cristatus, larva 34 mm. long. The ventro-lateral aspect of the cartilaginous ear capsule. The cephalic part of the fenestra is occupied by the small and partially fused columella above, a growth of cartilage below. The operculum is still broadly joined to the ear capsule.

Fig. 28. Typhlomolge rathbuni, adult. Lateral aspect of the ear region. Attention is particularly called to the segmented (fragmented) stilus columellæ and its relation to the facial nerve (ramus jugularis).

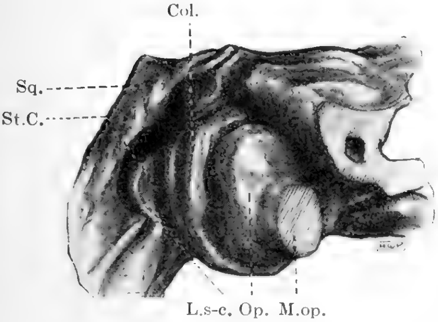

FIG. 25.

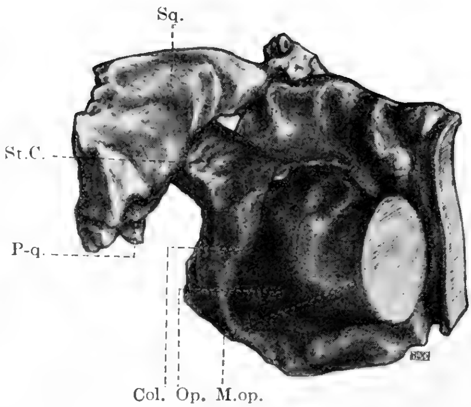

FIG. 26.

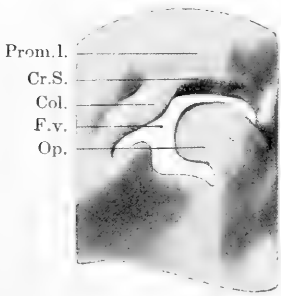

FIG. 27.

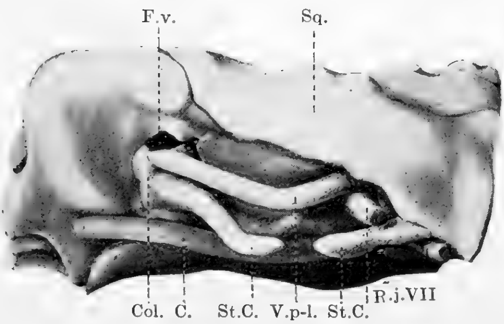

FIG. 28.

EXPLANATION OF PLATES III-IX.

A series of microphotographs to illustrate particularly the morphological relations of columella and operculum, from sections through the ear region.

PLATE III.

Fig. 29. Ambystoma punctatum, adult. Section through the stilus columellae, showing the columellar ligament and its relation to vein and facial nerve.

FIG. 30. The same. Section farther caudad. The fused columella is external to the cephalic end of the operculum.

Fig. 31. Ambystoma punctatum, larva, early transformation. There is shown columella and the floor of the ear capsule to become operculum. Compare with Fig. 32.

Fig. 32. Ambystoma punctatum, transformation period. The operculum is, at this level, separated from the floor of the ear capsule.

Fig. 33. Ambystoma punctatum, larva, 35 mm. in length. It shows the columella, stilus, squamoso-columellar ligament. Note the relation of the vein and artery in this and the two preceding figures.

Fig. 34. Chondrotus tenebrosus, larva. Note the massive stilus articulating with the squamosum and its relation to artery, vein and nerve.

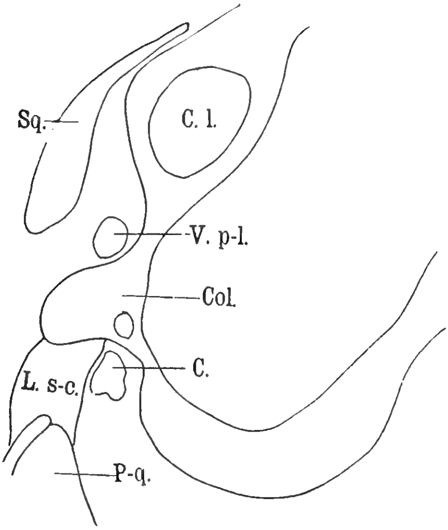

FIG. 29.—Adult Ambystoma.

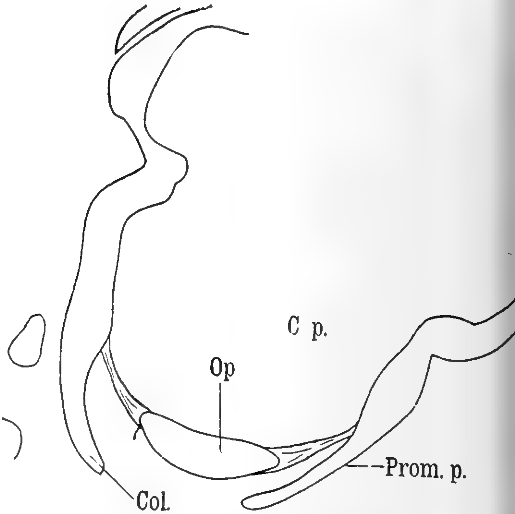

FIG. 30.—Adult Ambystoma.

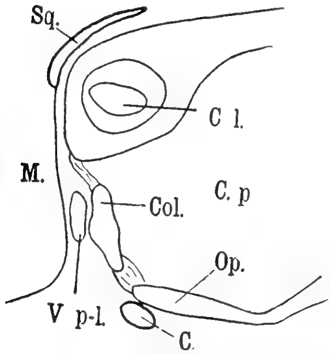

FIG. 31.—Larval Ambystoma.

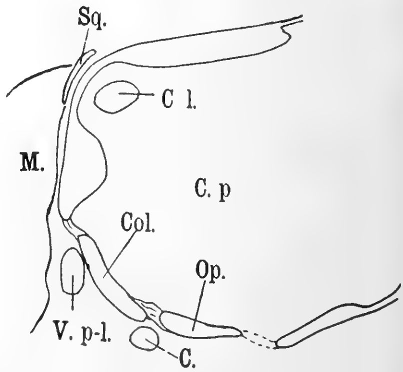

FIG. 32.—Larval Ambystoma.

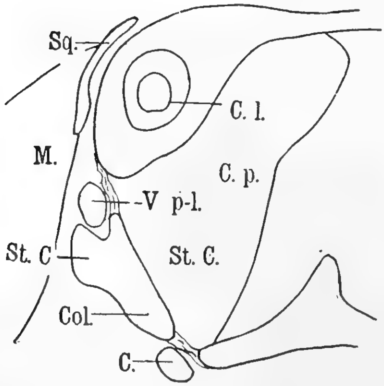

FIG. 33.—Larval Ambystoma.

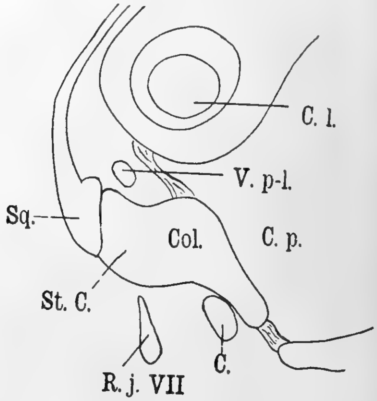

FIG. 34.—Larval Chondrotus.

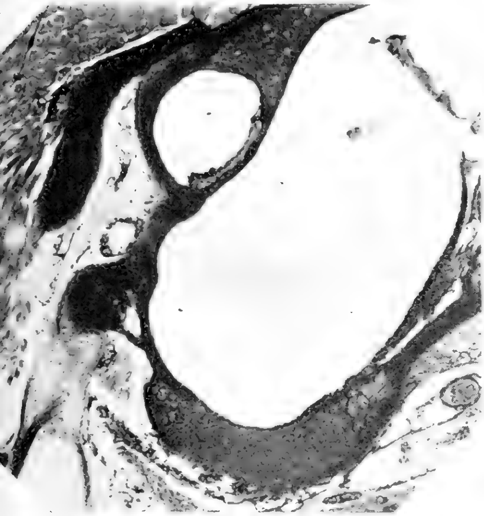

Fig. 29.—Adult Ambystoma.

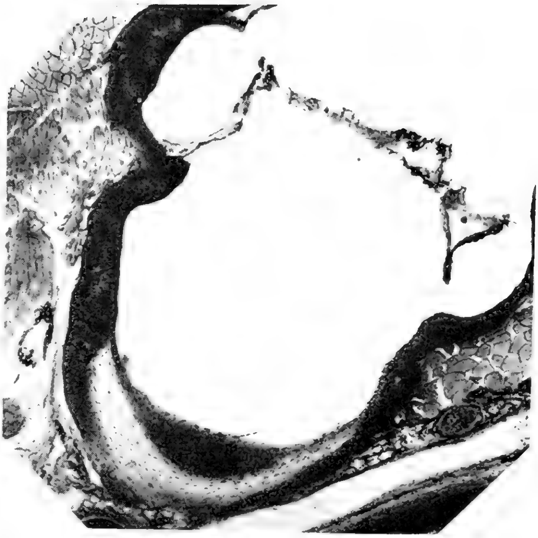

Fig. 30.—Adult Ambystoma.

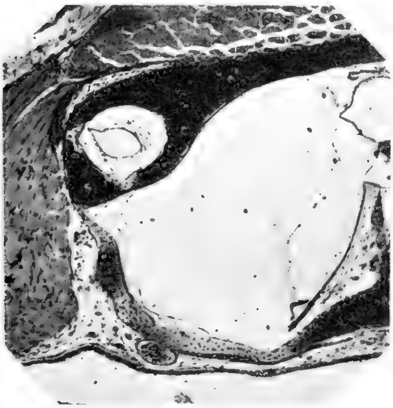

Fig. 31.—Larval Ambystoma.

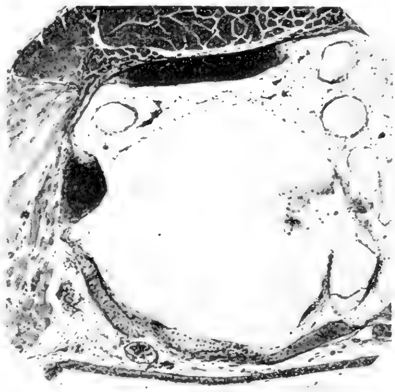

Fig. 32.—Larval Ambystoma.

Fig. 33.—Larval Ambystoma.

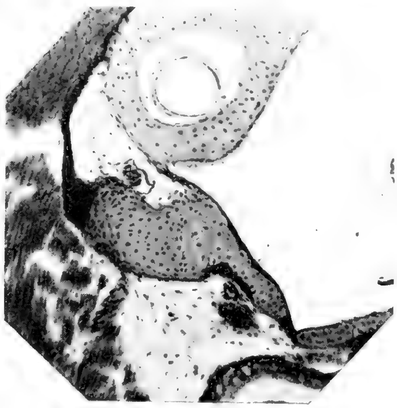

Fig. 34.—Larval Chondrotus.

Plate IV.

Fig. 35. Ambystoma punctatum, adult. Section farther caudad than Fig. 30. Operculum, opercular muscle and recessus perilymphaticus are shown.

Fig. 36. Chondrotus tenebrosus, transformation period. Section through stilus columellæ and the cephalic end of the developing operculum.

Fig. 37. Ambystoma punctatum, transforming. Horizontal section showing columella, stilus, squamoso-columellar ligament, the developing operculum and the M. opercularis attached in front to the opercular plate and behind to the suprascapula.

Fig. 38. Chondrotus tenebrosus, transformation period. Section farther caudad than Fig. 36. It shows the columella. The operculum is partially cut out from the floor of the ear capsule.

Fig. 39. Ambystoma punctatum, embryo 13-14 mm. in length. The columella consists of a mass of cells against the membrane of the fenestra and connected by a strand of dense tissue with the cells upon the prominence of the lateral semicircular canal. Above the columellar proton is the vena petroso-lateralis; below, the carotid artery.

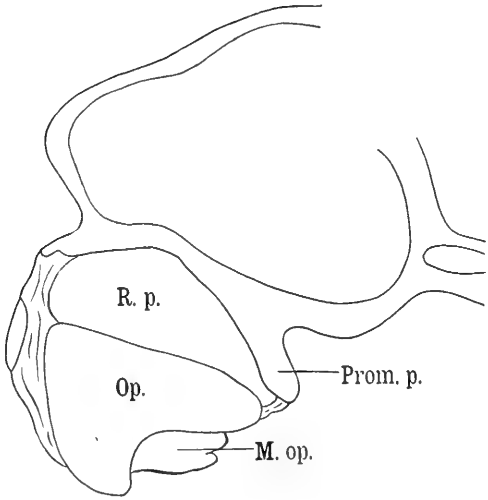

Fig. 35.—Adult Ambystoma.

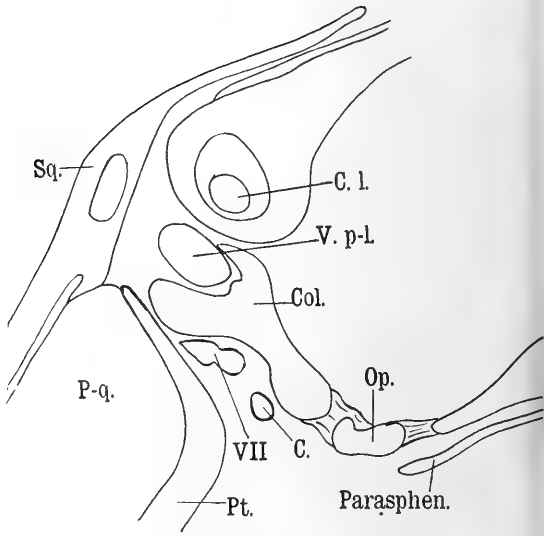

Fig. 36.—Transforming Chondrotus.

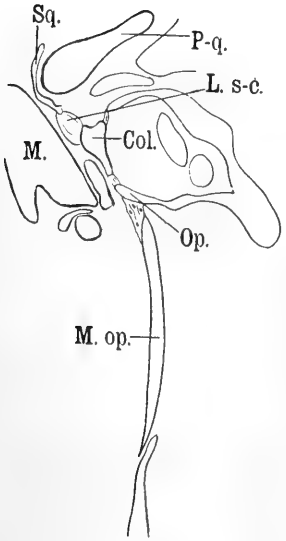

Fig. 37.
Transforming Ambystoma.

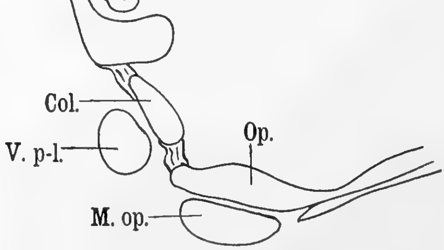

Fig. 38.—Transforming Chondrotus.

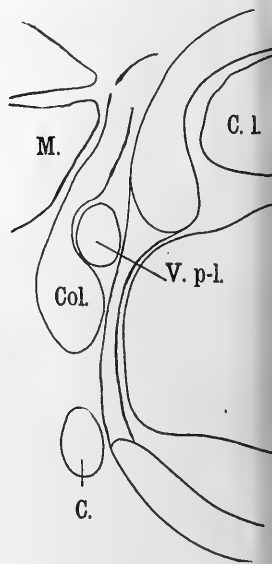

Fig. 39.
Embryo Ambystoma.

FIG. 35.—Adult Ambystoma.

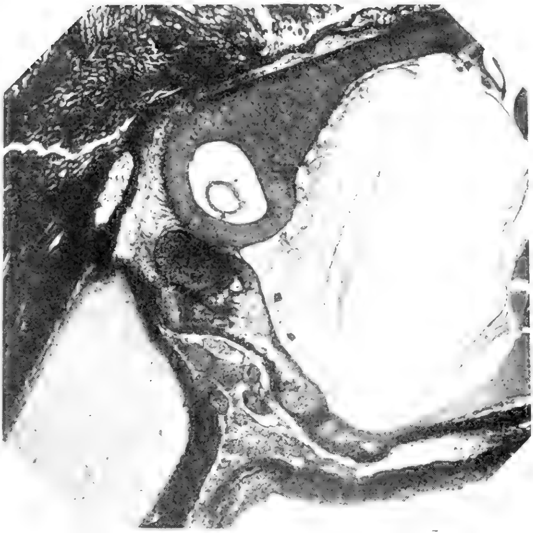

FIG. 36.—Transforming Chondrotus.

FIG. 37.
Transforming Ambystoma.

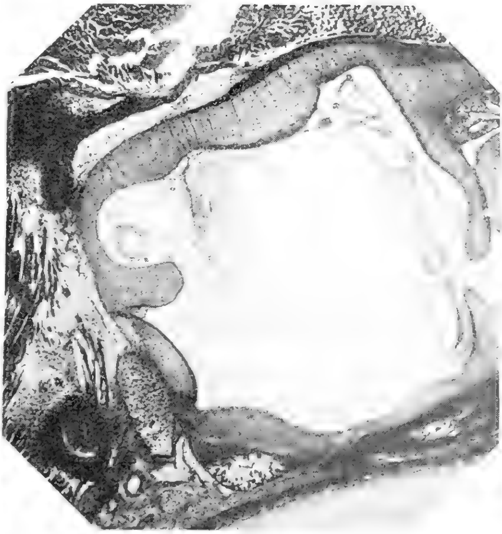

FIG. 38.—Transforming Chondrotus.

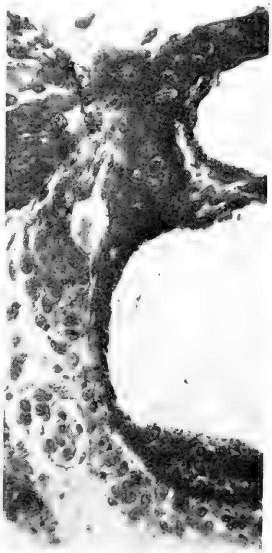

FIG. 39.
Embryo Ambystoma.

PLATE V.

Fig. 40. Salamandra maculosa, adult. Section through the stilus columellæ which is continuous with the palatoquadrate.

Fig. 41. The same. Section farther caudad, through the cephalic end of the operculum.

Fig. 42. The same. Section farther caudad through the operculum and the recessus. The M. opercularis would be shown in a section farther caudad.

Fig. 43. Triton cristatus, adult. Section through the cephalic portion of the operculum.

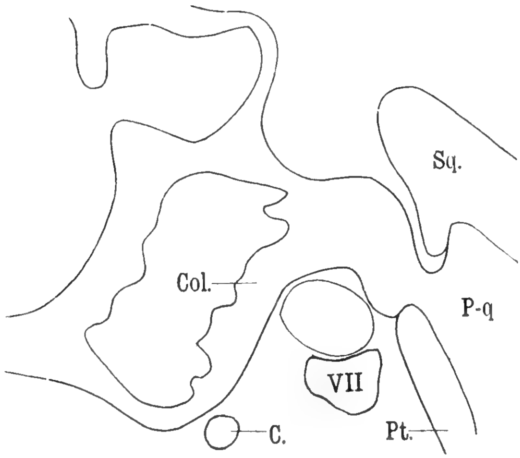

FIG. 40.—Adult Salamandra.

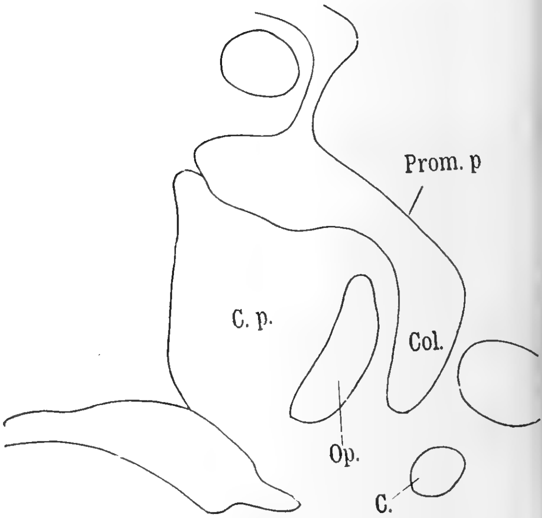

FIG. 41.—Adult Salamandra.

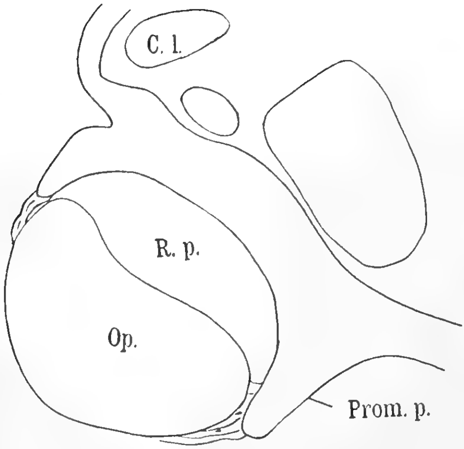

FIG. 42.—Adult Salamandra.

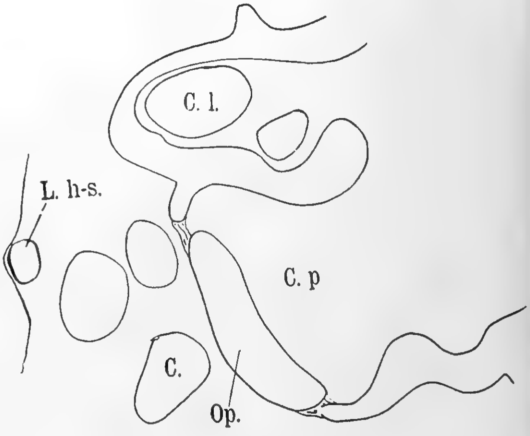

FIG. 43.—Adult Triton.

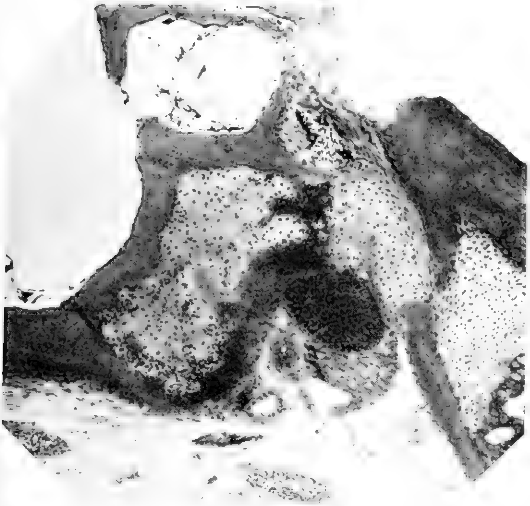

FIG. 40.—Adult Salamandra.

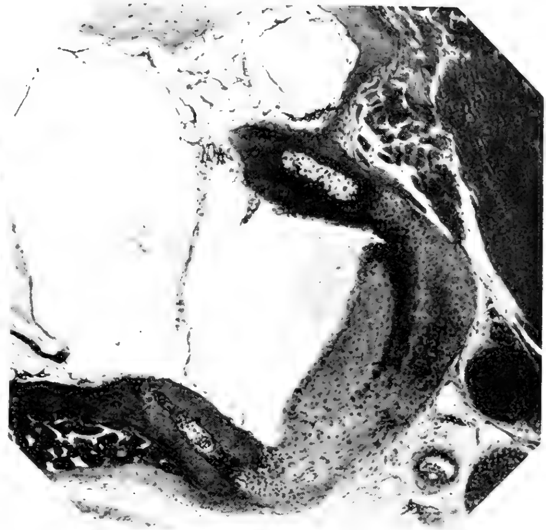

FIG. 41.—Adult Salamandra.

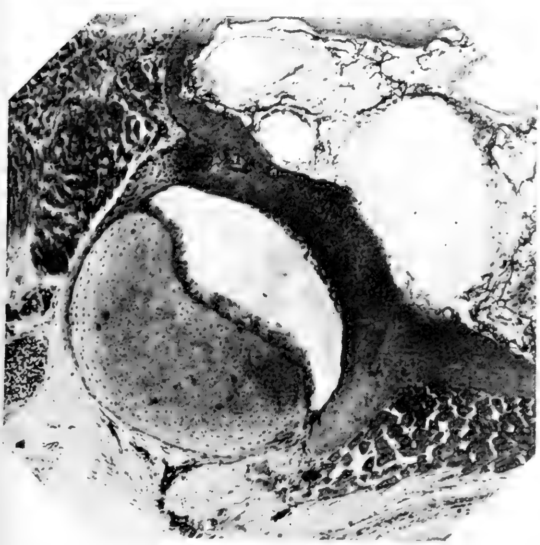

FIG. 42.—Adult Salamandra.

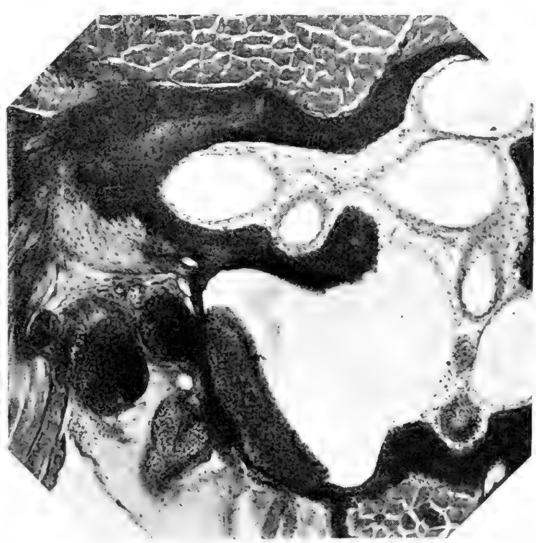

FIG. 43.—Adult Triton.

Plate VI.

Fig. 44. The same. Section farther caudad, showing the operculum, M. opercularis and recessus perilymphaticus.

Fig. 45. Gyrinophilus porphyriticus, adult. Section through the cephalic end of the fenestra vestibuli and the distal end of the stilus columellæ showing its articulation. Note the relation of blood vessels and facial nerve.

Fig. 46. The same. Section through the base of the stilus, a few sections farther caudad.

Fig. 47. The same. Several sections farther caudad, through the caudal portion of the fenestral plate, opercular muscle and recessus.

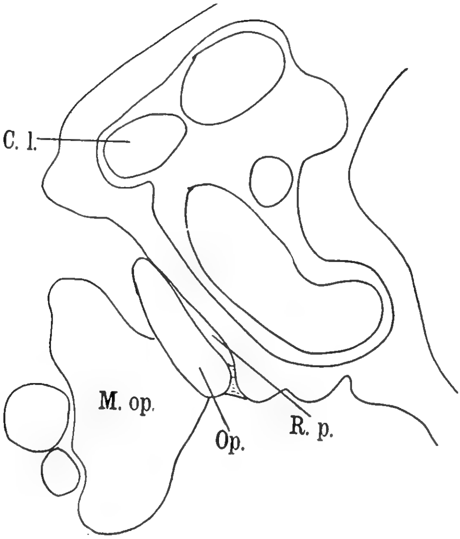

FIG. 44.—Adult Triton.

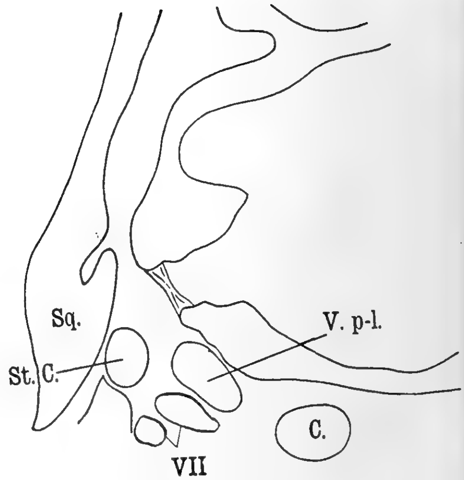

FIG. 45.—Adult Gyrinophilus.

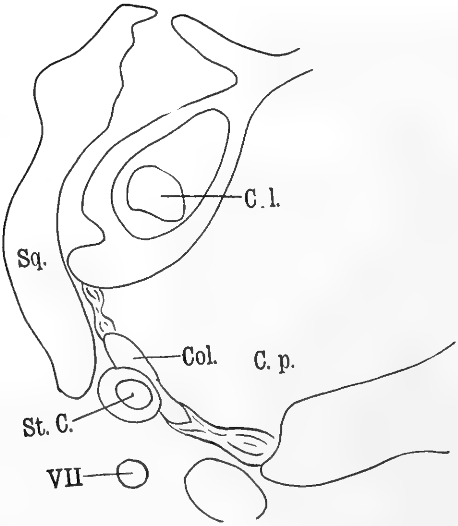

FIG. 46.—Adult Gyrinophilus.

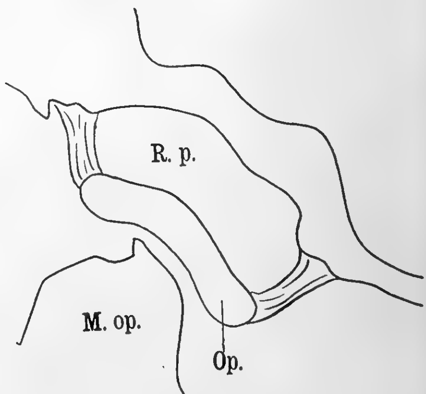

FIG. 47.—Adult Gyrinophilus.

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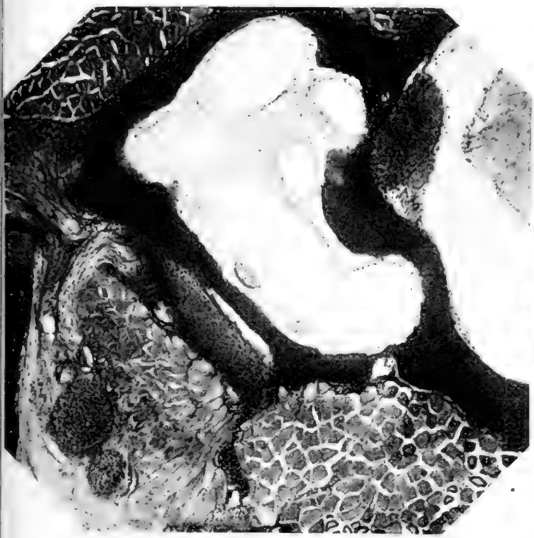

Fig. 44.—Adult Triton.

Fig. 45.—Adult Gyrinophilus.

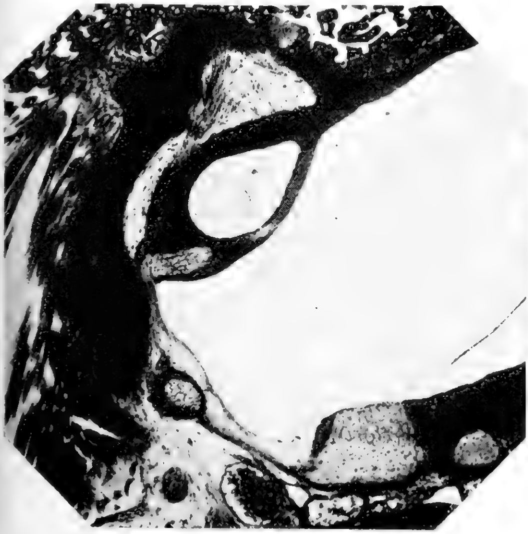

Fig. 46.—Adult Gyrinophilus.

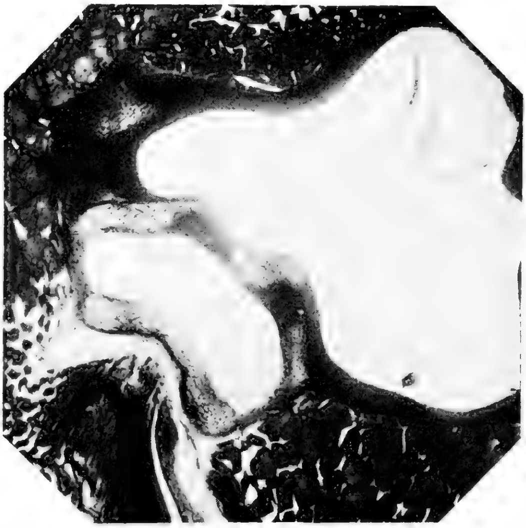

Fig. 47.—Adult Gyrinophilus.

Plate VII.

Fig. 48. Triton cristatus, larva 36 mm. long. The section is through the cephalic end of the columella where it is continuous with the ear capsule on its medial side.

Fig. 49. The same, farther caudad, through the columella and just in front of the cephalic end of the operculum.

Fig. 50. The same. A section still farther caudad, through the operculum and back of the caudal end of the columella. Note the relations of the blood vessels in this and the two preceding figures.

Fig. 51. Cryptobranchus allegheniensis, larva 34 mm. long. The ligamentum hyo-columellare is just joining the stilus at its bend.

Fig. 52. Crytobranchus allegheniensis. Just hatched larva. The proton of the columella is seen between the artery and vein.

Fig. 53. The same. A section farther cephalad to show the proton of the squamosal connection of the columella.

Fig. 54. Cryptobranchus allegheniensis, adult. A section through the distal end of the stilus to show its articulation.

Fig. 55. The same. A section farther caudad showing the columella and its large stilus illustrating the relation to artery, vein and nerve.

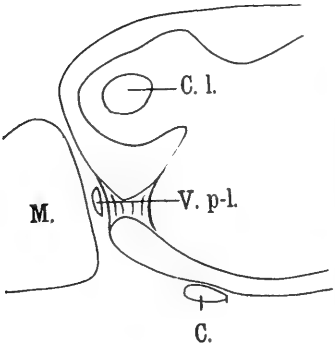

FIG. 48.—Larval Triton.

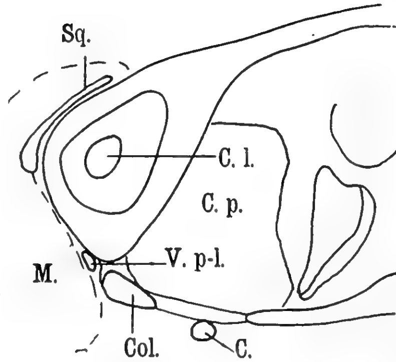

FIG. 49.—Larval Triton.

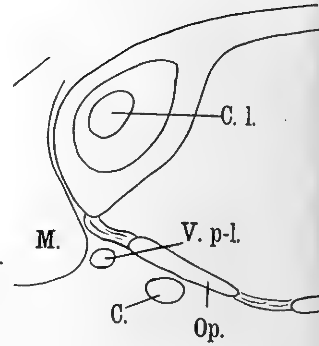

FIG. 50.—Larval Triton.

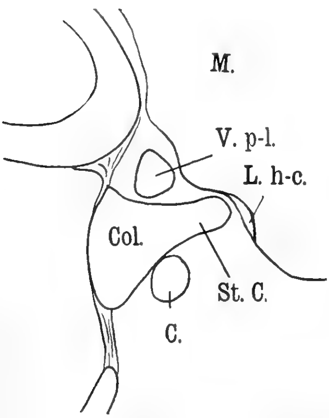

FIG. 51.
Larval Cryptobranchus.

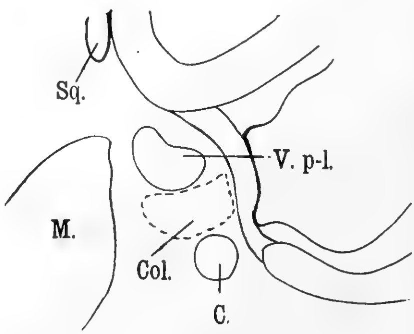

FIG. 52.—Just hatched Cryptobranchus.

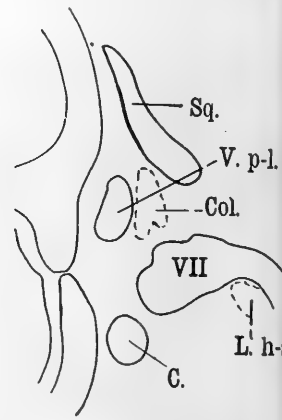

FIG. 53.
Just hatched Cryptobranchus

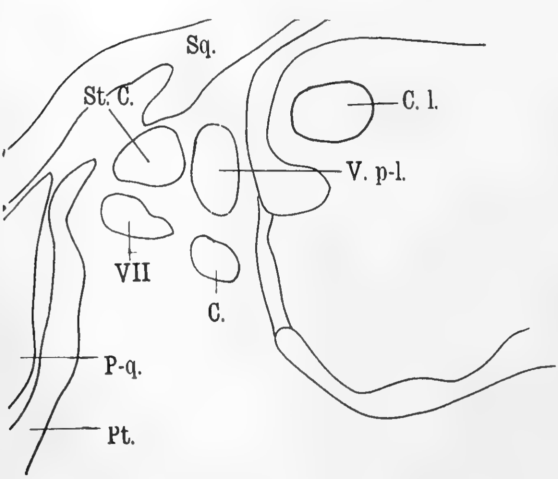

FIG. 54.—Adult Cryptobranchus.

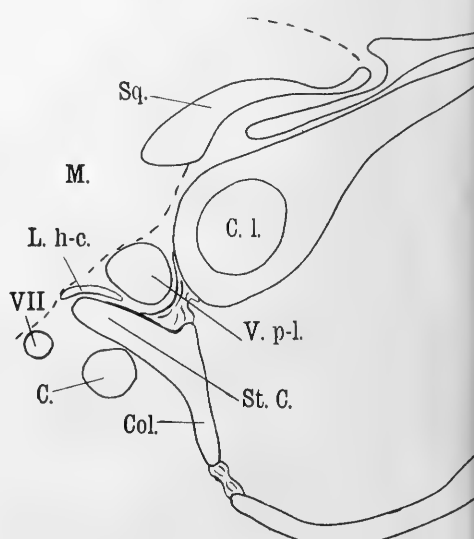

FIG. 55.—Adult Cryptobranchus.

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Fig. 48.—Larval Triton.

Fig. 49.—Larval Triton.

Fig. 50.—Larval Triton

Fig. 51.
Larval Cryptobranchus.

Fig. 52.—Just hatched Cryptobranchus.

Fig. 53.
Just hatched Cryptobranchus.

Fig. 54.—Adult Cryptobranchus.

Fig. 55.—Adult Cryptobranchus.

Plate VIII.

Fig. 56. The same. A few sections farther caudad through the caudal portion of the fenestral plate.

Fig. 57. Necturus maculosus, larva. The ligamentum squamoso-columellare is just attaching to the ventral edge of the squamosum.

Fig. 58. The same, a few sections farther caudad. The ramus jugularis VII is shown passing over the ligament.

Fig. 59. The same, still farther caudad through the base of the stilus columellæ. Note the relations of artery, vein and nerve in this and the two preceding figures.

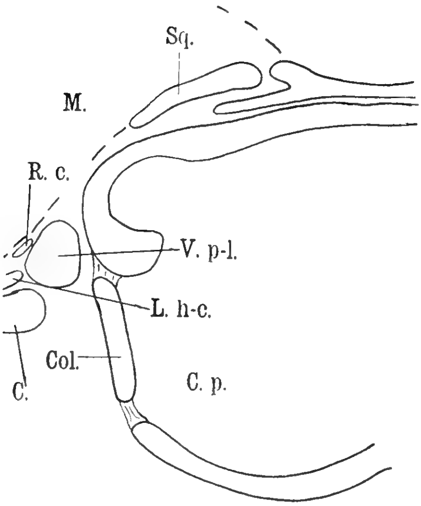

Fig. 56.—Adult Cryptobranchus.

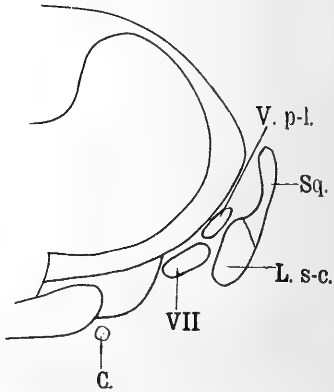

Fig. 57.—Larval Necturus.

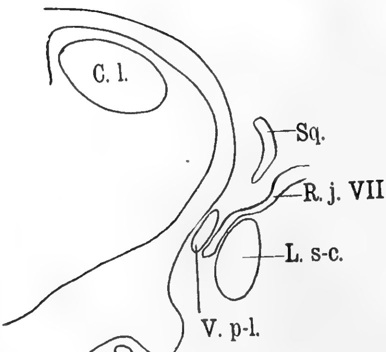

Fig. 58.—Larval Necturus.

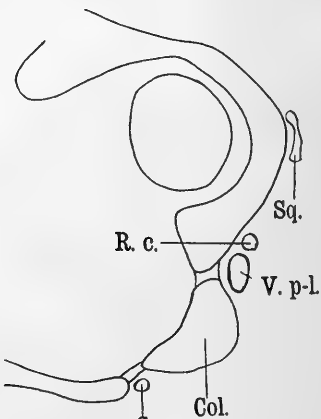

Fig. 59.—Larval Necturus.

FIG. 56.—Adult Cryptobranchus.

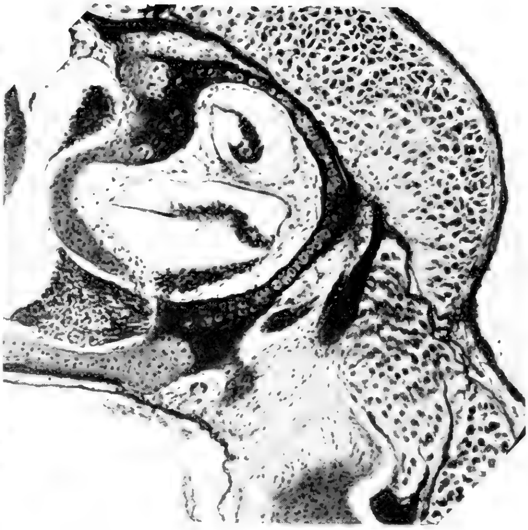

FIG. 57.—Larval Necturus.

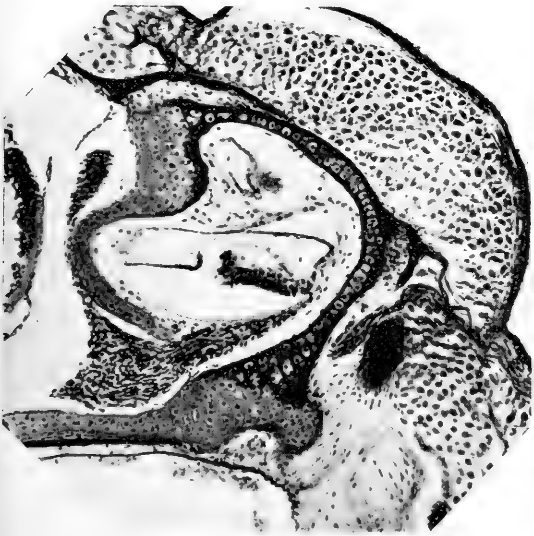

FIG. 58.—Larval Necturus.

FIG. 59.—Larval Necturus.

Plate IX.

Fig. 60. The same, still farther caudad, showing the caudal portion of the fenestral plate of the columella with its inner and outer bony plates.

Fig. 61. Siren lacertina, adult. Section showing the columella, ceratohyale the hyo-columellar ligament, into which projects the short stilus, and the cartilaginous portion of the ear capsule that forms the perilymphatic prominence.

Fig. 62. The same, a few sections farther caudad. The caudal end of the stilus is in the ligament. The perilymphatic prominence; position of artery and vein.

Fig. 63. The same, farther caudad behind the fenestra and showing the caudal portion of the perilymphatic prominence.

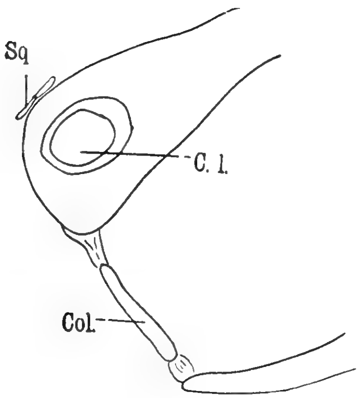

FIG. 60.—Larval Necturus.

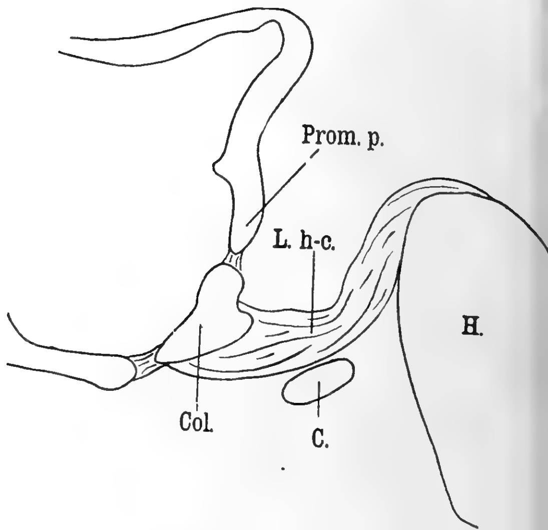

FIG. 61.—Adult Siren.

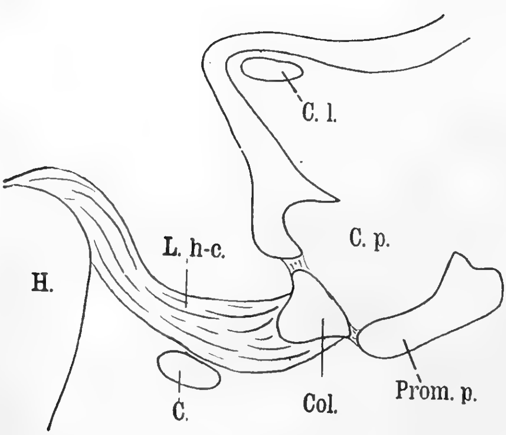

FIG. 62.—Adult Siren.

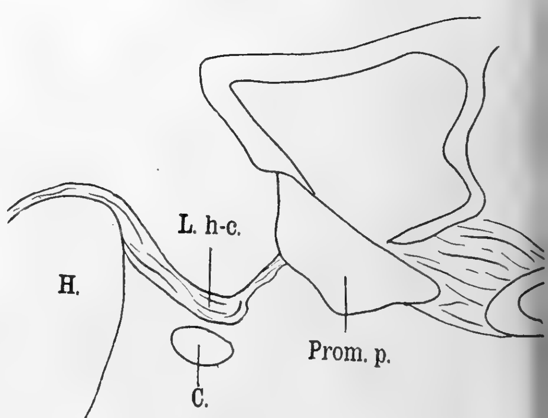

FIG. 63.—Adult Siren.

FIG. 60.—Larval Necturus.

FIG. 61.—Adult Siren.

FIG. 62.—Adult Siren.

FIG. 63.—Adult Siren.

Plate X.

(Figures 64-72)

A series of schemas showing the number and relative position of the elements present in the fenestra vestibuli in the various groups of the tailed Amphibia, together with the relation of these elements to the ear capsule, suspensorium, hyoid arch, M. opercularis, and facial nerve. It should be stated that they are to show relations only; the representation of the processes of the palatoquadrate, for example, being purely diagrammatic. In Fig. 67 the relation of the hyoid arch (H.) is intended to represent the condition in certain forms only.

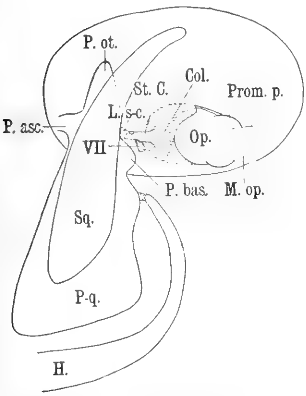

FIG. 64.—Ambystoma.

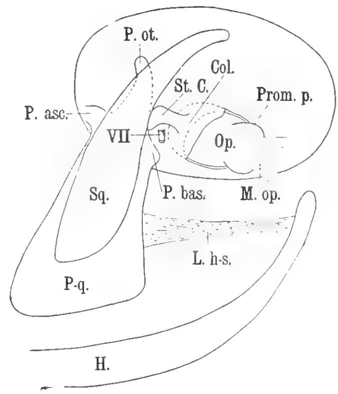

FIG. 65.—Salamandra.

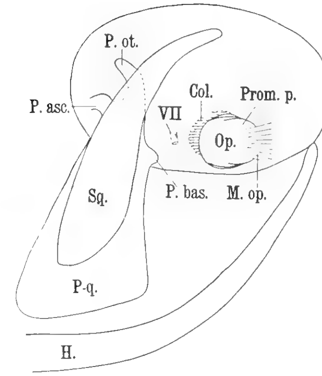

FIG. 66.—Triton and Diemictylus.

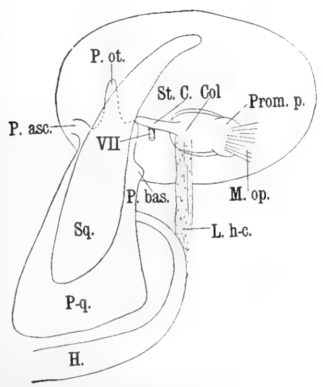

FIG. 67.—Plethodontidæ and Desmognathidæ.

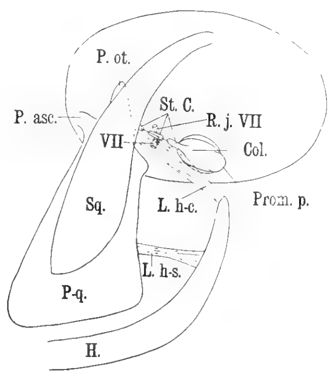

FIG. 68.—Typhlomolge.

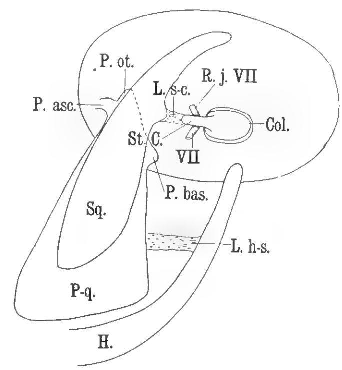

FIG. 69.—Necturus.

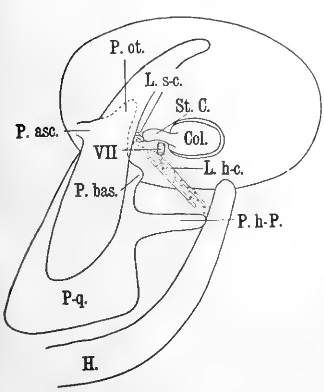

FIG. 70.—Cryptobranchus.

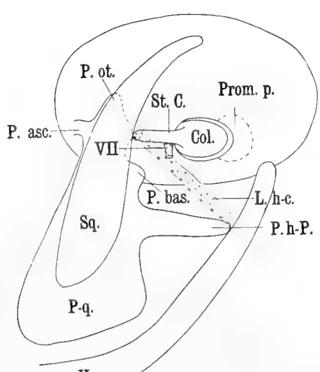

FIG. 71.—Amphiuma.

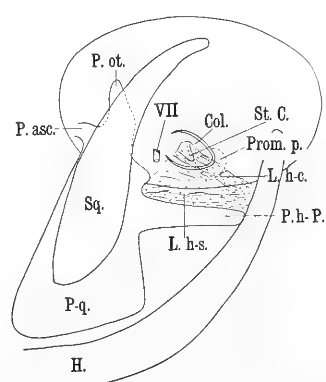

FIG. 72.—Siren.